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**RESEARCH ARTICLE**

# Phyto-Chemical Studies and *In vitro* Free Radical Scavenging Activity of *Swietenia mahagoni* (L.) Jacq.

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## ABSTRACT

Phyto-chemical screening and free radical scavenging activity of *Swietenia mahagoni* at Pachaimalai Hills of Eastern Ghats, Tamil Nadu, India, has been studied. Field study was carried out over a period of one year. On *In vitro* studies of *S. mahagoni*, the air dried powdered leaf, stem bark and fruit were extracted successively in soxhlet extractor with acetone, methanol and water. Phyto-chemical analysis for alkaloids, flavonoids, saponins, phenols, steroids, tannins, carbohydrates, proteins, amino acids and glycosides were examined qualitatively and quantitatively. Free radical scavenging activity was evaluated through DPPH method. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, steroids and sterols, saponin, tannins, phenolic compounds, glycosides, carbohydrate, protein and amino acids.The methanol extraction was more efficient where most of the said phytochemicals were present DPPH radical scavenging activity of different solvent extracts of leaves, stem bark and fruits of *S. mahagoni* at five different concentrations (20-100µg) in the reaction mixture. All the extracts exhibited dose dependent increase in activity. The concentration of the sample extracts required to decrease initial concentration of DPPH by 50% ($IC_{50}$) under experimental condition has been calculated.

**Key words:** *Swietenia mahagoni* , DPPH assay, free radical scavenging activity, phytochemical.

# INTRODUCTION

Medicinal plants which constitute a segment of the flora in biodiversity provide raw material for use in all the indigenous systems of medicine. According to the World Health Organization (WHO), 80% of the population in developing countries relies on traditional medicine, mostly in the form of plant derivatives to the extent of about 25%. Numerous drugs have entered the international pharmacopeia via the study of ethnopharmacology and ethnobotany. Ethnopharmacology and ethnobotany are interdisciplinary fields of research that looks specifically at the empirical knowledge of indigenous people concerning medicinal substances, their potential health benefits on their health risks associated with such remedies. As can be seen, many of the plant-derived pharmaceuticals and phytomedicines currently in use were used by native people around the world. Some of this knowledge has been documented and codified or studied scientifically. Also of the hundreds of thousands of species of living plants, only a fraction has been investigated in the laboratory.

There is growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine [1].The present study is focused on the indigenous medicinal plant *Swietenia mahagoni* (L.) Jacq. (Meliaceae), which is also known as *Mahagoni* tree. It has been used to reduce hypertension, diabetes, inflammation, arthritis, malaria, and epilepsy.Despite several ethnobotanical and ethnopahrmacological surveys on the therapeutic use of *Swietenia* species, a systematic pharmacological evaluation of the *S. mahagoni* is lacking.

Based on the above, the present work was carried out with the following objectives; to assess the preliminary phytochemical constituents of different solvent extract of leaf, stem bark and fruit of *Swietenia mahagoni* and to evaluate the antioxidant and free radical scavenging potential of different solvent extract of leaf, stem bark and fruit of *S. mahagoni*.

# MATERIALS AND METHODS

## Collection and authentication of plant material

Fresh leaves, stem bark and fruits of *Swietenia mahagoni* (L.) Jacq. (Meliaceae) were collected during the month of November, 2011 from Pachamalai hills of Eastern Ghats, Tamil Nadu, India. The plant material was identified and its authenticity confirmed by comparing with the voucher specimen at the herbarium of Botanical Survey of India, Southern Circle, Coimbatore, India (Fig.1)

## Shade drying of the collected leaf material

Freshly collected leaf, stem bark and fruits were cleaned to remove adhering dust and then dried under shade. The dried plant materials were powdered in a Willy Mill to 60-mesh size. The leaf, stem bark and fruits powder was used for further studies.

## Solvent extraction

The air dried powdered plant materials of *S. mahagoni* were successively extracted in soxhlet extractor with acetone and methanol. Each time, before extracting with the next solvent, the powdered material was dried in hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 h and the water extract filtered. All the extracts were evaporated to remove even the final traces of the respective solvents. The percentage yields were expressed in terms of the air dried drug. All the solvent extracts were used for the *in vitro* studies. For *in vivo* studies, the shade dried and powdered leaf material was extracted in soxhlet extractor with ethanol after dewaxing with petroleum ether. The extract was evaporated to remove even the final traces of ethanol. The dried extract was suspended in distilled water right before use.

### *In vitro* studies

### Qualitative phytochemical evaluation

Phytochemical screening of different solvent extracts was carried out different methods [2 & 3].

#### Tests for alkaloids

Dragendroff's reagent: To 1 mL of the extract, 1 mL of Dragendroff's reagent was added. The appearance of orange red precipitate indicates the presence of alkaloids.

#### Tests for flavonoids

Shinoda test: To 1 mL of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.

#### Tests for steroids and sterols

Salkowski's test: The extract was dissolved in 2mL of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer-turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterol compounds, in the extract.

#### Tests for tannins and phenolic compounds

a. To 1 mL of the extract, few mL of 5% neutral ferric chloride was added. The development of a dark bluish black colour indicates the presence of tannins.
b. To 1 mL of the extract, few mL of lead tetra acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.
c. A small quantity of the extract was dissolved in 0.5 mL of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue in the presence of phenols.

#### Test for saponins (Foam test)

About 1 mL of alcoholic extract was diluted with 20 mL of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.

#### Tests for carbohydrates

a. Fehling's test: Five mL of Fehling's solution was added to 2 mL of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.
b. Iodine test: Two mL of dilute iodine solution was added to the extract. The appearance of blue colour indicates the presence of starch.

#### Test for protein and amino acids

a. Biuret test: To 1 mL of extract, equal volume of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added. The appearance of violet colour indicates the presence of proteins.
b. Ninhydrin test: To the extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

### Determination of total phenolics and tannins

The total phenolic content of different solvent extracts of *S. mahagoni* leaves, stem bark and fruit was determined [4]. Ten microlitre aliquots of the extracts (10mg/2ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were

placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.Using the same extracts the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) [5]. One hundred milligrams of PVPP was weighed into a 100×12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4°C for 4h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows; Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

**Determination of total flavonoid content**

The flavonoid content of different solvent extracts of *S. mahagoni* leaves, stem bark and fruit was determined by the use of a slightly modified colorimetry method [6]. A 0.5ml aliquot of appropriately (10mg/2ml) diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

### Determination of *in vitro* antioxidant activity

### Free radical scavenging activity on DPPH

The antioxidant activity of the different solvent extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH [7]. The sample extracts at various concentrations (20-100 µg) was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Gallic acid and BHA were used as positive control. Percentage radical scavenging activity of the sample was calculated as follows;

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition ($IC_{50}$) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

## RESULTS AND DISCUSSION

### Phytochemical screening

To investigate the chemical constituents of *S. mahagoni* leaves, stem bark and fruits, the successive solvent extracts were subjected to qualitative phytochemical screening. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, steroids and sterols, saponin, tannins, phenolic compounds, glycosides, carbohydrate, protein and amino acids. The methanol extraction was more efficient where most of the said phytochemicals were present (Table 1).

### DPPH radical scavenging activity of *S. mahagoni*

The DPPH radical is a stable organic free radical which has been extensively used for evaluating the free radical scavenging potential of natural antioxidants. Table 2 and Fig. 2. Illustrate the DPPH radical scavenging activity of different solvent extracts of leaves, stem bark and fruits of *S. mahagoni* at five different concentrations (20-100µg) in the reaction mixture. All the extracts exhibited dose dependent increase in activity. The concentration of the sample extracts required to decrease initial concentration of DPPH by 50% ($IC_{50}$) under experimental condition has been

calculated. A lower $IC_{50}$ indicates higher antioxidant activity. The higher DPPH radical scavenging activity was shown by methanol extract of the stem bark with an $IC_{50}$ of 24.76 ± 0.09µg/ml followed by the water extract (26.65 ± 0.07µg/ml) of leaf powder. Water extract of the fruit powder showed the lowest DPPH radical scavenging activity with an $IC_{50}$ of 124.74 ± 0.90 µg/ml. However, all the tested extracts exhibited lesser hydrogen donating ability than the positive standards gallic acid ($IC_{50}$ 3.40 ± 0.32 µg/ml) and BHA ($IC_{50}$ 5.20 ± 0.26 µg/ml).

## CONCLUSSION

In this study free radical scavenging activity was evaluated through DPPH method. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, steroid and sterol, saponin, tannis, phenolic compounds, glycosides, corpohydrate, protein and amino acids. The qualitative phytochemical evaluation of different solvent extracts of *S. mahagoni* reveals the presence of various compounds. However the components responsible for the radical scavenging activities of the extracts are known. Therefore future research is needed for the isolation and identification of the active components in the extracts.

## REFERENCES

1. Misar AV Kale M Joshi M and Mujumdar AM. Analgesic Activity of *Dalbergia lanceolaria* barks extract in Swiss Albino Mice. *Pharmaceutical Biology*, 2005; 43(8): 723-725.
2. Harborne JB. Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd Edition, London, New York, 1984.
3. Kokate CK. In: practical pharmacognosy, 4th edition. Vallabh Prakashan, Delhi, 1994.
4. Siddhuraju P and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry*, 2003; 51: 2144-2155.
5. Siddhuraju P and Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) verdc.) seeds. *Food Chemistry*, 2007; 105: 950-958.
6. Zhishen J Mengcheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 1999; 64: 555- 559.
7. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, 1958; 26: 1199-1200.

**Figure 1. *Swietenia mahagoni* (L.) Jacq. Plant.**

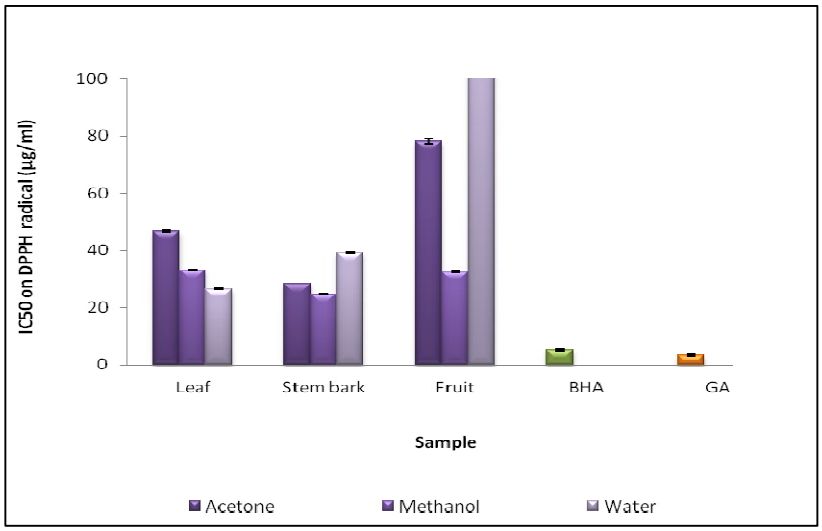


**Figure 2. $IC_{50}$ of different solvent extracts of *S. mahagoni* leaf, stem bark and fruit on DPPH radical**
Values are means of three independent analyses ± standard deviation (n = 3). BHA - Butylated hydroxyanisole; GA - Gallic acid.

**Table 1 .Qualitative phytochemical evaluation of different solvent extracts of *S. mahagoni*.**

| Sample | Extract | Phytochemical tests | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Alkaloids | Flavonoids | Saponins | Phenols | Steroids | Tannins | Carbohydr-ates | Protein & Amino acids | Glycosides |
| Leaf | Acetone extract | + | + | + | + | - | + | - | - | + |
| | Methanol extract | + | + | + | + | + | + | + | - | + |
| | Water extract | + | + | - | + | + | + | + | + | + |
| Stem bark | Acetone extract | + | + | - | + | + | + | - | - | + |
| | Methanol extract | + | + | + | + | + | + | - | - | + |
| | Water extract | - | + | + | + | - | + | + | + | + |
| Fruit | Acetone extract | + | + | - | + | + | + | - | + | - |
| | Methanol extract | + | + | - | + | + | + | + | + | + |
| | Water extract | + | + | + | - | + | + | + | + | - |

'+' - Presence of compounds; '–' – Absence of compounds.

**Table 2 .DPPH radical scavenging activity of different solvent extracts of *S. mahagoni*.**

| Solvent | Concentration (µg) | Percentage activity | | |
|---|---|---|---|---|
| | | Leaf | Stem bark | Fruit |
| **Acetone extract** | 20 | 9.62 ± 0.51 | 15.52 ± 1.08 | 5.56 ± 0.26 |
| | 40 | 18.48 ± 0.53 | 29.17 ± 0.49 | 10.61 ± 0.19 |
| | 60 | 26.85 ± 0.91 | 42.37 ± 0.18 | 15.57 ± 0.32 |
| | 80 | 36.96 ± 0.58 | 56.68 ± 0.25 | 20.28 ± 0.46 |
| | 100 | 45.98 ± 0.62 | 70.37 ± 0.30 | 25.41 ± 0.46 |
| **Methanol extract** | 20 | 12.95 ± 0.17 | 16.35 ± 0.24 | 12.38 ± 1.27 |
| | 40 | 24.72 ± 0.86 | 33.98 ± 1.21 | 26.05 ± 0.69 |
| | 60 | 35.46 ± 0.97 | 48.05 ± 0.30 | 36.24 ± 1.24 |
| | 80 | 49.21 ± 0.97 | 64.32 ± 0.61 | 49.95 ± 1.00 |
| | 100 | 59.96 ± 0.77 | 80.60 ± 0.30 | 60.88 ± 0.80 |
| **Water extract** | 20 | 15.47 ± 0.57 | 9.83 ± 0.54 | 3.68 ± 0.12 |
| | 40 | 31.32 ± 0.56 | 20.27 ± 0.83 | 7.07 ± 0.38 |
| | 60 | 45.36 ± 0.36 | 30.68 ± 1.21 | 9.52 ± 0.12 |
| | 80 | 59.78 ± 0.45 | 51.58 ± 1.61 | 13.21 ± 0.33 |
| | 100 | 74.53 ± 0.45 | 50.84 ± 0.63 | 15.58 ± 0.26 |

Values are means of three independent analyses ± standard deviation (n = 3).

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**SHORT COMMUNICATION**

# Partial Mitochondrial DNA Sequence and Amino Acid Analysis of the Cytochrome C Oxidase Subunit I Gene from *Rhynocoris marginatus* (Fabricius) (Heteroptera: Reduviidae).

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## ABSTRACT

The partial nucleotide sequence of the assassin bug *Rhynocoris marginatus* (Fabricius), mitochondrial cytochrome c oxidase subunit I gene has been identified. The nucleotide sequence codes for a 218 amino acid sequences. The highest A+T rich composition in *R. marginatus* COI gene is particularly biased at the first codon position, which totaled to 25.2%. The *R. marginatus* COI gene sequence shows high homology, up to 86% identity, with several other insect gene sequences and a phylogenetic analysis indicates that the *R. marginatus* COI gene sequence is closely related to three other *Rhynocoris* species, *R. fuscipes* (Fabricius), *R. longifrons* (Stål) and *R. kumarii* Ambrose and Livingstone. Hydropathy plot of the *in-silico* translated amino acid sequence of the *R. marginatus* COI gene protein indicates more of hydrophilic residues (denoted by the peaks) and less of hydrophobic residues. Totally 218 frequency of codons observed from *R. marginatus* COI gene nucleotide sequence through the invertebrate mitochondrial translation table. PCR analysis showed that COI gene is expressed at different level in nucleotide and amino acid sequences.

**Key words:** *R. marginatus*, COI, Mitochondrial gene, Phylogeny.

*Rhynocoris marginatus* is a predominant and potential reduviid predator of many economically important pests in India. The COI gene nucleotide sequences reported in this paper have been submitted to GenBank and have been assigned the accession numbers: *R. marginatus* GQ229415, *R. fuscipes* GQ229414, *R. kumarii* GQ229413 and *R. longifrons* GQ229412. Comparative studies of mitochondrial DNA (mtDNA) among different species have revealed an overall well conserved organization across metazoan but significant differences also exist. For example, compared to vertebrate mtDNA, insect mtDNA is much more A+T rich [4, 5, 18]. The study of mtDNA also has led to the discovery that several genetic codes, differing from the universal code, are used in animal mitochondria that vary among vertebrates, echinoderms, insects, nematodes and cnideria [28]. Mitochondria genes have frequently been used as molecular markers in evolutionary studies [7, 26, 29]. Indeed, mitochondrial genes are often sequences of choice for phylogenetic studies as they are (1) highly conserved among phyla, (2) maternally inherited, (3) present in high copy number and (4) became mtDNA evolves faster than nuclear DNA [16].

Cytochrome c oxidase subunit I (COI) is the terminal catalyst in the mitochondrial respiratory chain and is involved in electron transport and proton translocation across the membrane [22, 9]. COI gene is the mitochondrial marker often used for evolutionary study became (1) it is the largest of the three mitochondrial-encoded cytochrome oxidase subunits [4, 2] and (2) the protein sequence contains highly conserved functional domains and variable regions [22, 9].

We report here the isolation of the partial mitochondrial DNA sequence of the assassin bug, *R. marginatus*, cytochrome c oxidase subunit I gene. The COI gene was identified following random nucleotide sequencing of mtDNA inserts from an assassin bug. The preparation of the mtDNA was described elsewhere [23, 1]. *R. marginatus* cytochrome c oxidase subunit I gene is 657 nucleotides in length and encodes a 218 amino acid sequence. The partial nucleotide sequence and its deduced amino acid sequence are shown in Figure 2. Hydropathy plot of the *in-silico* translated amino acid sequence of the *R. marginatus* COI gene protein indicates more of hydrophilic residues (denoted by the peaks) and less of hydrophobic residues (Figure 1), in accordance with the topographical model of the COI protein [22]. In the *R. marginatus* COI gene, the putative initiation codon is TTT and the termination codon is a triple TTT at final position 657. These suggested codons are consistent with those reported elsewhere [2, 14]. The translation start codon in insects remains unclear, although in *Drosophila* species, the tetra-nucleotide ATAA is usually recognized as the start codon [6].

In *R. marginatus*, as for *Rhynocoris* species, the TTT is preceded by the hexanucleotide ATTTAA and it is unlikely that it serves as the initiation start. Therefore, our results agree with the suggestion by [2] that the TTT is used as an initiation codon in dipterans, and it seems likely that exact codon may vary across insect species. The A+T percentage for the *R. marginatus* COI gene is 61.9% and G+C percentage is 38.1%. The analysis also revealed the nucleotide frequencies of A-28.9%, T-33.1%, C-20.2% and G-17.8% (Table 1). The COI gene sequence of *R. marginatus* that were assessed indicated an overall rich A+T% which correlated well with earlier reports on insect under the family of Reduviidae [17, 21, 13]. This also agrees with the general nature of A+T rich genome in insects [25, 24].

The codon usage in *R. marginatus* COI gene, as seen table 2, clearly shows a preference for A or T ending codons. The base composition of mtDNA is highly correlated with codon usage, because insect mitochondrial protein genes exhibit a preference for using A+T rich codons [5]. The phenomena seem characteristic of the insect, while the A+T content are significantly lower in crustaceans than in insects [7]. Totally 218 frequency of codons observed from *R. marginatus* COI gene nucleotide sequence through the invertebrate mitochondrial translation table (Table 2). Because of the codon preference, the base composition in *R. marginatus* COI gene is particularly biased at the first codon position, which totaled 25.2% A+T (Table 1). The A+T content at the second and third positions are 17.79 and 18.87 respectively. The similar variation observed in the A+T% is also a characteristic of insects under the order of Hymenoptera. Earlier reports indicate higher A+T content than the average (80%) in the family of Apidae. However, the reverse was observed in Coleoptera insects (69.8%) [12].

The COI gene was isolated for four different *Rhynocoris* species, such as *R. marginatus*, *R. fuscipes*, *R. longifrons* and *R. kumarii* using primers flanking the 5′- and 3′- ends (LCO1490F 5′- GGTCAACAAATCAAAGATATTGG -3′& HCO2198R 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′)[21]. With alignment of *R. fuscipes* nucleotide and amino acid sequences as consensus, it was observed that there were 147 and 37 positions of nucleotide and amino acid substitutions respectively in *R. marginatus*. All nucleotide substitutions are base transitions and occur as synonymous substitutions and, therefore, the *R. marginatus* COI gene is identical for the four *Rhynocoris* species (data not shown). The results are consistent with others. Higher levels of variation at synonymous sites are commonly observed, because most of these mutations are not functional constrained [18, 26].

The *R. marginatus* COI gene sequence was submitted to the BLAST program (http:// www.ncbi.nlm.nih.gov/) for homology searches. *R. marginatus* COI gene is 86% identical to *Pselliopus coccinea* COI (AY252970), 84% to *Rhynocoris ventralis* COI (JQ888677) and 84% to *Rhynocoris segmentarius* COI (JQ888666). COI gene sequences reported in the literature are numerous; therefore several sequences were selected that provide a broad representation of the different insect genera and these sequences were aligned with *R. marginatus* COI using ClustalW [27]. The SMART analysis of *R. marginatus* COI gene sequence of 218 amino acid residues revealed four transmembrane segments between amino acid 42-64, 85-107, 127-149 and 162-184 in the sequence region connected by five coils (data not shown). The transmembrane segments from reports also revealed similar domains for the same region studied in *Drosophila melanogaster* Meigen and five transmembrane segments for *Triatoma rubida* Uhler COI gene [11, 21]. However, seven transmembrane segments were reported for this region in *Anopheles gambiae* Giles [15].

The phylogeny was constructed based on the aligned COI gene sequences and is shown in Figure 3. The phylogenetic relationships revealed the existence of two main clusters and a single sub-cluster. The population of *R. marginatus* formed one cluster (A) and populations of *R. fuscipes*, *R. longifrons* and *R. kumarii* grouped together in to the other cluster (B). In the cluster (B) the populations of *R. kumarii* and *R. longifrons* formed one sub-cluster. There were a total of 593 positions in the final dataset. The optimal tree with the sum branch length of 0.35 is shown (Figure 3) and all the Phylogeny was generated with MEGA4. The Phylogeny of the COI gene generated in this study matched with the sequence information result that is already reported in *Rhodnius neivai* Lent and *Triatoma maculata* Erichson (Gaunt and Miles, 2002) and *Oncerotrachelus* sp. and *Cylapus* sp. [23].

To test the universality of the conserved primers identified in this study, PCR amplifications have been carried out for four *Rhynocoris* species populations. Agarose gel electrophoresis of the PCR amplicon resulted in a strong band at all approximately 750 bp for all the DNA samples (Figure 4). Mitochondrial genes have been studied to find the genetic information that may be useful for investigating molecular phylogeny and evolution. The present study was to be compared the nucleotide and amino acid sequences of the mitochondrial COI gene from economically important *R. marginatus* with an objective to develop molecular markers important for examining the molecular genetic variation and also to understand the deep phylogenetic relationships of the *Rhynocoris* species.

As recent studies have shown that mitochondrial COI gene participates in the apoptotic process [3] and it is known that, for example, *Plasmodium* parasites, the causal agent of malaria, induce severe damage to the mosquito midgut epithelium [10]. Indeed, COI enzyme activity is over expressed in praziquantel-resistant strain of *Schistosoma mansoni* [20] and COI expression levels increase in the late stages of infection of *Bombyx mori* cells infected with nucleopolyhedovirus (BmNPV) [19].

Thus, the present study is an initiation attempt which throws light and also provides some preliminary ideas on the variation in the COI gene sequence of the *R. marginatus*. In addition the variation also observed across four different *Rhynocoris* species were sufficient to group the *Rhynocoris* species in to different clusters. However, further studies employing other *Rhynocoris* species from different location would throws light on the utility of COI gene sequence to determine phylogenetic relationships among them.

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## REFERENCES

1. Baskar A. Molecular genetic variation and phylogeny of *Rhynocoris* spp. based on mitochondrial gene analysis (Heteroptera: Reduviidae), Ph.D., thesis. University of Madras, Tamil Nadu, India; 2010.
2. Beard CB, Hamm DM, Collins FH. The mitochondrial genome of the mosquito *Anopheles gambiae*: DNA sequence, genome organization, and comparisons with mitochondrial sequences of other insects. Insect Molecular Biology 1993; 2: 103-124.
3. Bossy-Wetzel E, Newmeyer DD, Green DR. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. Journal of EMBO 1998; 17: 37-49.
4. Clary DO, Wolstenholme DR. The mitochondrial DNA molecular of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. Journal of Molecular Evolution 1985; 22: 252-271.
5. Crozier RH, Crozier YC. The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 1993; 133: 97-117.
6. De Bruijn MH. *Drosophila melanogaster* mitochondrial DNA, a novel organization and genetic code. Nature 1983; 304: 234-241.
7. Garcia-Machado E, Pempera M, Dennebouy N, Oliva-Suarez M, Mounolou JC, Monnerot M. Mitochondrial genes collectively suggest the paraphyly of Crustacea with respect to Insecta. Journal of Molecular Evolution 1999; 49, 142-149.
8. Gaunt MW, Miles MA. An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Molecular Biological Evolution 2002; 19: 748-761.
9. Gennis RB. Site-directed mutagenesis studies on subunit I of the aa3-type cytochrome c oxidase of *Rhodobacter sphaeroides*: a brief review of progress to date. Biochimica et Biophysica Acta 1992; 1101: 184-187.
10. Han YS, Thompson J, Kafatos FC, Barillas-Mury C. Molecular interactions between *Anopheles stephensi* midgut cells and *Plasmodium berghei*: the time bomb theory of ookinete invasion of mosquitoes. Journal of EMBO 2000; 19: 6030-6040.
11. Hill E, Broadbent ID, Chothia C, Pettitt J. Cadherin superfamily proteins in *Caenorhabditis elegans* and *Drosophila melanogaster*. Journal of Molecular Biology 2001; 305: 1011-1024.
12. Jermiin LS, Crozier RH. The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufonigra*: sequence divergence in Hymenoptera is associated with nucleotide content. Journal of Molecular Evolution 1994; 38: 282-294.
13. Liu H, Zhao P, Li S, Cai W. Taxonomic status of *Velinoides matsumure* (Hemiptera: Reduviidae) inferred from mitochondrial and nuclear genes. *Zootaxo* 2009; 55-68.
14. Lunt DH, Zhang DX, Szymura JM, Hewitt GM. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Molecular Biology 1996; 5: 153-165.
15. Moita C, Simoes S, Moita F, Jacinto A, Fernandes P. The cadherin superfamily in *Anopheles gambiae*: a comparative study with *Drosophila melanogaster*. Comparative Functional Genomic 2005; 204-206.
16. Moriyama EN, Powell JR. Synonymous substitution rates in *Drosophila*: mitochondrial versus nuclear genes. Journal of Molecular Evolution 1997; 45: 378-391.
17. Muraji M, Kawasaki K, Shimizu T. Nucleotide sequence variation use of mitochondrial DNA for phylogenetic analysis in Anthocorid Bugs (Hemiptera: Anthocoridae). *JARQ* 2001; 35: 85-90.

18. Navajas M, Fournier D, Lagnel J, Gutierrez J, Boursot P. Mitochondrial COI sequences in mites: evidence for variations in base composition. Insect Molecular Biology 1996; 5: 281-285.
19. Okano K, Shimada T, Mita K, Maeda S. Comparative expressed-sequence-tag analysis of differential gene expression profiles in BmNPV-infected BmN cells. Virology 2001; 282: 348-356.
20. Pereira C, Fallon PG, Cornette J, Capron A, Doenhoff MJ, Pierce RJ. Alterations in cytochrome c oxidase expression between praziquantel-resistant and susceptible strains of *Schistosoma mansoni*. Parasitology 1998; 117: 63-73.
21. Pfeiler E, Bitler BG, Ramsey JM, Palacios-Cardiel C, Markow TA. Genetic variation, population structure and phylogenetic relationships of *Triatoma rubida* and *T. recurva* (Hemiptera: Reduviidae) from the Sonoran Desert, insect vectors of the chagas disease parasite *Trypanosoma cruzi*. Molecular Phylogenetic Evolution 2006; 209-221.
22. Saraste M. Structural features of cytochrome oxidase. Quarterly Reviews of Biophysics 1990; 23: 331-366.
23. Schuh RT, Weirauch C, Wheeler WC. Phylogenetic relationships within the cimicomorpha (Hemiptera: Heteroptera), a total evidence analysis. Systematic Entomology 2009; 34: 15-48.
24. Sperling FAH, Byers JR, Hickey DA. Mitochondrial DNA sequence variation among pheromotypes of the dingy cutworm, *Feltia jaculifera* Gn. (Lepidoptera: Noctuidae). Canadian Journal of Zoology 1996; 74: 2109-2119.
25. Sperling FAH, Hickey DA. Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura* spp; Lepidoptera). Molecular Biological Evolution 1994; 11: 656-665.
26. Stahls G, Nyblom K. Phylogenetic analysis of the genus *Cheilosia* (Diptera, Syrphidae) using mitochondrial COI sequence data. Molecular Phylogenetic and Evolution 2000; 15: 235-241.
27. Thompson JD, Higgins, DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 1994; 22: 4673-4680.
28. Wolstenholme DR. Animal mitochondrial DNA: structure and evolution. International Review of Cytology 1992; 141: 173-216
29. Wu W, Schmidt TR, Goodman M, Grossman, LI. Molecular evolution of cytochrome c oxidase subunit I in primates: is there coevolution between mitochondrial and nuclear genomes?.Molecular Phylogenetic and Evolution 2000; 17: 294-304.

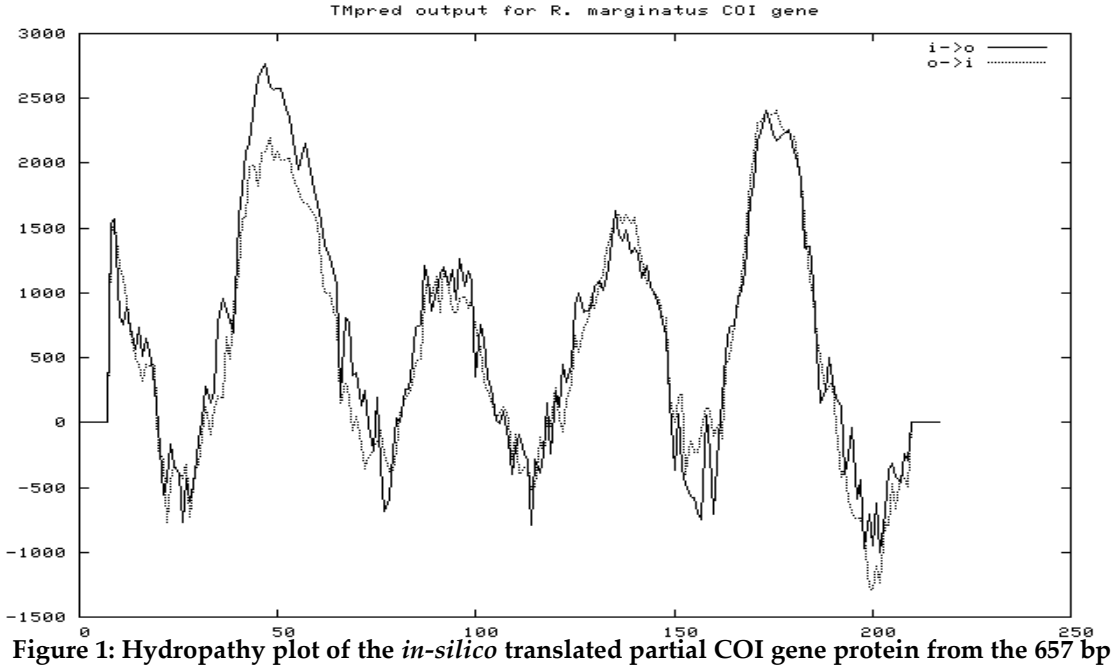


**Figure 1: Hydropathy plot of the *in-silico* translated partial COI gene protein from the 657 bp nucleotide sequence from *R. marginatus*.** (Predicted by TMpred at http://www.ch.embnet.org).

```
TTT CTT ACT GGG TTC CTG AGC AGG AGT ATT AGG GAC TTC GCT GAG ATG AAT AAT TCG TAT  60
 F   L   L   G   S   W   A   G   V   L   G   T   S   L   S   W   M   I   R   I   20

TGA ATT GGG AAC ACC AGG TAC ATT TAT TGG AAA TGA CCA AAT TTA TAA CGT ATT CGT TAC 120
 E   L   G   T   P   G   T   F   I   G   N   D   Q   I   Y   N   V   F   V   T   40

TGC TCA TGC ATT TAT TAT AAT TTT CTT CAT AGT TAT ACC TAT TAT AAT CGG AGG GTT CGG 180
 A   H   A   F   I   M   I   F   F   M   V   M   P   I   M   I   G   G   F   G   60

TAA CTG ATT AGT GCC ATT AAT AAT TGG AGC CCC TGA CAT GGC ATT TCC ACG AAT AAA TAA 240
 N   W   L   V   P   L   M   I   G   A   P   D   M   A   F   P   R   M   N   N   80

CAT AAG ATT TTG ACT TCT ACC CCC ATC CCT CAC ACT TCT ACT AGT AAG TAG TAT TGC AGA 300
 M   S   F   W   L   L   P   P   S   L   T   L   L   L   V   S   S   I   A   E  100

AGG GGG GGC GGG AAC AGG ATG AAC TGT GTA CCC TCC ATT ATC TAG AAA TAT AGC TCA TAG 360
 G   G   A   G   T   G   W   T   V   Y   P   P   L   S   S   N   M   A   H   S  120

AGG AGC ATC CGT TGA CTT AGC CAT CTT CTC ATT ACA TTT AGC AGG CGT ATC ATC AAT TTT 420
 G   A   S   V   D   L   A   I   F   S   L   H   L   A   G   V   S   S   I   L  140

AGG AGC AGT AAA TTT CAT TTC AAC TAT CAT TAA TAT ACG ACC CGT GGG TAT AAC TCC TGA 480
 G   A   V   N   F   I   S   T   I   I   N   M   R   P   V   G   M   T   P   E   60

ACG AAT CCC GCT ATT TGT ATG ATC AGT AGC AAT TAC TGC ATT ACT TCT CTT CTT ATC ACT 540
 R   I   P   L   F   V   W   S   V   A   I   T   A   L   L   L   F   L   S   L  180

CCC AGT ATT AGC CGG AGC AAT TAC CAT ACT ACT AAC GGA TCG AAA TTT TAA TAC TTC ATT 600
 P   V   L   A   G   A   I   T   M   L   L   T   D   R   N   F   N   T   S   F  200

CTT TGA CCC AGT AGT AGG TGG AGA TCC AAT CCT GTA TCA ACA TTT ATT CTG ATT TTT     657
 F   D   P   V   V   G   G   D   P   I   L   Y   Q   H   L   F   W   F         218
```

**Figure 2: The 657 bp nucleotide sequence and conceptually translated amino acid sequence of the PCR amplicon of the COI gene from *R. marginatus* (GenBank accession number GQ229415).**

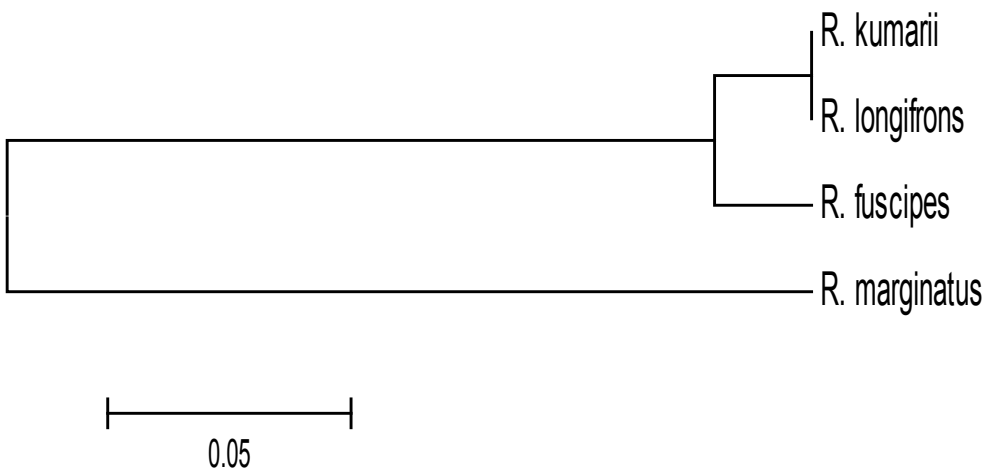


**Figure 3: Phylogenetic relationships of the four *Rhynocoris* species based on the nucleotide sequence of the PCR amplicon 750 bp of the COI gene, derived from Neighbour-joining algorithm using MEGA software (version 3.1). Note:** The phylogeny revealed the existence of two main clusters and a single sub-cluster. The population of *R. marginatus* formed one cluster (A) and populations of *R. fuscipes*, *R. longifrons* and *R. kumarii* grouped together in to the other cluster (B). In the cluster (B) the populations of *R. kumarii* and *R. longifrons* formed one sub-cluster.

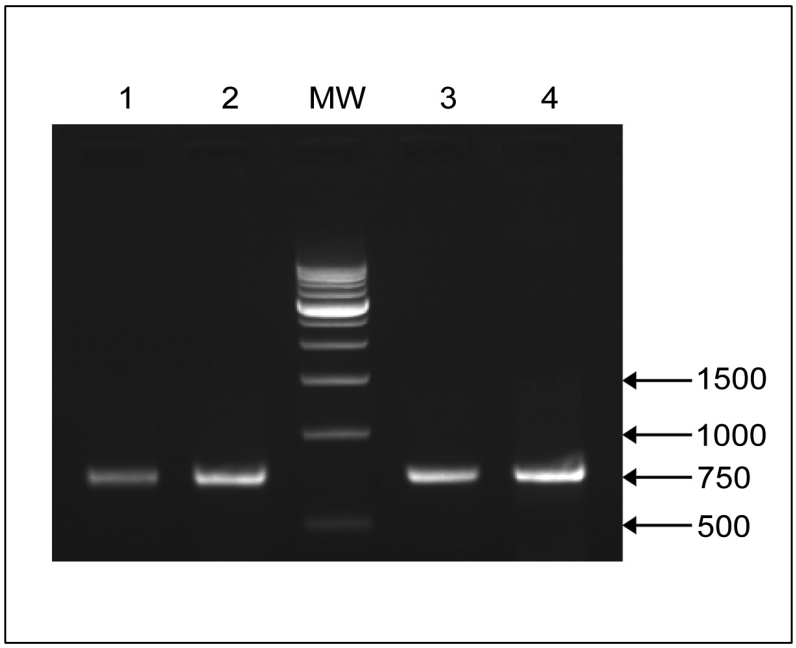


**Figure 4: Agarose gel electrophoresis (1.2%) of the 750 bp PCR amplicon of the COI partial gene from four different species of *Rhynocoris*: Lane 1, *R. marginatus*; Lane 2, *R. fuscipes*; Lane MW, 500 bp DNA ladder; Lane 3, *R. kumarii* and Lane 4, *R. longifrons*.**

**Table 1: Base composition in the 657 bp nucleotide sequence of the COI gene at the three codon positions in *R. marginatus*.** Note: *The highest A+T composition in *R. marginatus* is particularly biased at the first codon position, which totaled to 25.2%.

| Codon positions | A | T | C | G |
|---|---|---|---|---|
| **First position** | 15.37* | 9.83* | 5.63 | 2.40 |
| **Second position** | 9.27 | 8.52 | 6.39 | 9.43 |
| **Third position** | 4.26 | 14.61 | 8.21 | 6.08 |

**Table 2: Frequency of 218 codons in COI gene nucleotide sequence from *R. marginatus* (GenBank accession number GQ229415) and follow the invertebrate mitochondrial translation table.**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| gca | Ala(A) | 10 | gaa | Glu(E) | 3 | aag | Lys(K) | 0 | uaa | Ter(.) | 0 |
| gcc | Ala(A) | 3 | gag | Glu(E) | 0 | --- | Lys(K) | 0 | uag | Ter(.) | 0 |
| gcg | Ala(A) | 1 | --- | Glu(E) | 3 | aua | Met(M) | 12 | --- | Ter(.) | 0 |
| gcu | Ala(A) | 2 | gga | Gly(G) | 11 | aug | Met(M) | 1 | aca | Thr(T) | 4 |
| --- | Ala(A) | 16 | ggc | Gly(G) | 1 | --- | Met(M) | 13 | acc | Thr(T) | 1 |
| cga | Arg(R) | 4 | ggg | Gly(G) | 4 | uuc | Phe(F) | 10 | acg | Thr(T) | 1 |
| cgc | Arg(R) | 0 | ggu | Gly(G) | 5 | uuu | Phe(F) | 8 | acu | Thr(T) | 7 |
| cgg | Arg(R) | 0 | --- | Gly(G) | 21 | --- | Phe(F) | 18 | --- | Thr(T) | 13 |
| cgu | Arg(R) | 1 | cac | His(H) | 0 | cca | Pro(P) | 8 | uga | Trp(W) | 7 |
| --- | Arg(R) | 5 | cau | His(H) | 4 | ccc | Pro(P) | 2 | ugg | Trp(W) | 0 |
| aac | Asn(N) | 3 | --- | His(H) | 4 | ccg | Pro(P) | 1 | --- | Trp(W) | 7 |
| aau | Asn(N) | 7 | auc | Ile(I) | 5 | ccu | Pro(P) | 4 | uac | Tyr(Y) | 1 |
| --- | Asn(N) | 10 | auu | Ile(I) | 14 | --- | Pro(P) | 15 | uau | Tyr(Y) | 2 |
| gac | Asp(D) | 4 | --- | Ile(I) | 19 | aga | Ser(S) | 4 | --- | Tyr(Y) | 3 |
| gau | Asp(D) | 2 | cua | Leu(L) | 6 | agc | Ser(S) | 0 | gua | Val(V) | 10 |
| --- | Asp(D) | 6 | cuc | Leu(L) | 3 | agg | Ser(S) | 0 | guc | Val(V) | 0 |
| ugc | Cys(C) | 0 | cug | Leu(L) | 3 | agu | Ser(S) | 2 | gug | Val(V) | 3 |
| ugu | Cys(C) | 0 | cuu | Leu(L) | 3 | uca | Ser(S) | 7 | guu | Val(V) | 3 |
| --- | Cys(C) | 0 | uua | Leu(L) | 13 | ucc | Ser(S) | 3 | --- | Val(V) | 16 |
| caa | Gln(Q) | 2 | uug | Leu(L) | 1 | ucg | Ser(S) | 1 | nnn | ???(X) | 0 |
| cag | Gln(Q) | 0 | --- | Leu(L) | 29 | ucu | Ser(S) | 1 | | | |
| --- | Gln(Q) | 2 | aaa | Lys(K) | 0 | --- | Ser(S) | 18 | | | |

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RESEARCH ARTICLE

# Evaluation of Hydroxyl Radical Scavenging Activity of Different Solvent Extract of *Euphorbia fusiformis* Buch.–Ham. ex. D.Don (Euphorbiaceae) Leaf and Rhizome.

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**ABSTRACT**

The present study was undertaken to evaluate the Hydroxyl radical scavenging activity of different solvent extract of *Euphorbia fusiformis* Buch.–Ham. ex. D.Don (Euphorbiaceae) (*Pal perukki kilangu in Tamil*) leaf and rhizome. For in vivo studies the powdered leaf and rhizome materials were extracted in soxhlet extractor with ethanol after dewaxing with petroleum ether.The scavenging activity of the different solvent extracts of *Euphorbia fusiformis* on hydroxyl radical was measured. These results support the notion that plant extract may have a many roles as pharmaceuticals.

**Keywords:** Hydroxyl radical scavenging activity, *Euphorbia fusiformis*, antioxidant.

## INTRODUCTION

According to world health organization medicinal plants would be the best source to obtain a variety of drugs[1]. Since ancient times, the use of medicinal plants is usually based on traditional knowledge from which their therapeutic properties are oftenly ratified in pharmacological studies [2].*Euphorbia fusiformis* (Pal perukki kilangu), Buch. –Ham. ex. D.Don (Euphorbiaceae) is a rare medicinal Plant found in Bengal [3]. Uttarpradesh[4], Konkan[5]. and central eastern ghats of tamil nadu [6]. In Gujarat state, it is found in Dangs, Rajpippala and chotaudaipur regions[7].The ethnobotanical value of this plant is due to its action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis[8],liver disorders and diarrhea[9].Thus the present study was carried out to evaluate the Hydroxyl radical scavenging activity of different solvent extract of *Euphorbia fusiformis*.

# MATERIALS AND METHODS

## Experimental studies

## Antioxidant studies

Reactive oxygen species damage the biological system and causes different chronic diseases like cancer and heart diseases[10].The modern research claims that oxidative stress is the cause of various disorders and diseases therefore, the researchers focus on the role of antioxidants in the maintenance of biological system (human health), its remedy and treatment [11].

**Free radicals**

Free radicals are defined as molecules having an unpaired electron in their outer orbit. They are generally unstable, highly reactive and energized molecules. Reactive oxygen species (ROS) can be classified into oxygen-centered radicals and oxygen-centered non-radicals. Oxygen-centered radicals are superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), alkoxyl radical ($RO^{\bullet}$), and peroxyl radical ($ROO^{\bullet}$). Oxygen-centered non-radicals are hydrogen peroxide ($H_2O_2$) and singlet oxygen ($O_2$). Other reactive species are nitrogen species such as nitric oxide ($NO^{\bullet}$), nitric dioxide ($NO_2^{\bullet}$), and peroxynitrite ($OONO^-$)[12,13].Reactive oxygen species in biological systems are related to free radicals, even though there are non-radical compounds in reactive oxygen species such as singlet oxygen and hydrogen peroxide.

## Determination of *in vitro* antioxidant activity

## Hydroxyl radical scavenging activity

The scavenging activity of the different solvent extracts of *E. fusiformis* on hydroxyl radical was measured according to the method of Klein *et al*. (1991)[14] 60-300µg of the extract were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4).The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. Gallic acid, BHA and the reaction mixture without sample were used as positive and negative control. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated as follows:

$$\%HRSA = (\text{control OD-sample OD} / \text{control OD}) \times 100$$

The analysis was performed in triplicate. The sample concentration providing 50% inhibition ($IC_{50}$) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

# RESULTS AND DISCUSSION

## Hydroxyl radical scavenging activity of *E. fusiformis*

Hydroxyl radical scavenging activity was estimated by generating hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radical formed by the oxidation, reacts with DMSO to yield formaldehyde, which provides a convenient method to detect hydroxyl radical by treatment with Nash reagent. In the present investigation, all the extracts of *E. fusiformis* generally exhibited strong hydroxyl radical scavenging activities (Table 1 and 2). Among them, the ethanol extract ($IC_{50}$ 54.40 ± 0.07 µg/ml) of the leaf showed higher level of hydroxyl radical scavenging activity (Fig.1). The $IC_{50}$ of the standard antioxidants BHA and GA were found to be 87.81 ± 2.42 µg/ml and 23.61 ± 0.18 µg/ml respectively.The hydroxyl radical is an extremely reactive free radical formed in biological systems and

has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells[15].This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition this species is considered to be one of the quick inhibitors of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids. To test the reaction of hydroxyl radical (•OH) with different solvent extract of *E. fusiformis* ascorbic acid-iron-EDTA was used to generate hydroxyl radical. The hydroxyl radical formed, by the oxidation reaction with dimethyl sulfoxide (DMSO) yields formaldehyde,which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent [16]. All the solvent extracts of leaf and rhizome exhibited hydroxyl radical scavenging activity (3.18% - 84.75%) at five different concentrations (60-300µg) in the reaction mixture (Table 1 and 2). Generally the higher levels of hydroxyl radical scavenging activity exhibited by ethanol extracts in the present study could be attributed to the active hydrogen donor ability of hydroxyl substitution provided by phenolic flavonoids extracted during ethanol extraction. Ethanol extract of pomegranate peel and grape fruit have also been reported to contain sustainable hydroxyl radical scavenging activity which might be attributed to the presence of phenolic substances [17]. Hagerman *et al*. (1998)[18] have also reported that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging activity by tannins with specific functional groups.

## CONCLUSION

From the results it may be concluded that the ability of different solvent extracts of *E. fusiformis* leaf and rhizome to quench hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of chain reaction. For that, further study for detailed investigation is needed.

## REFERENCES

1. Santos, FA, Cunha, GMA, Viana, G SB, Rao, VSN, Manoel, AN silveira ER Antibacterial activity of essential oils, from *Psidium* and *Pilocarpus* species of plants, Phytotheraphy research. 1995; 11(1):67 – 69.
2. Kirley M. Canuto, Edilberto R. Silveira, Antonio Marcos E. Bezerra, Luzia Kalyne A.M. Leal and Glauce Socorro B.Viana; Phytochemistry, Pharmacology and Agronomy of medicinal plants. *Amburana cearensis*, an interdisciplinary study. Empresa Brasieira de Pesquisa Agropercuaria, Universidade Federal do Ceara, Brazil. 354 – 374.
3. Duthie JF. Flora of the Upper Gangetic Plain and the Adjacent Siwalik and Sub-Himalayan Tracts. Vol.
4. Bishen Singh Mahendrapal Singh; New Connaught place, Dehradun: 1994. p. 72.
5. Bishen Singh Mahendrapal Singh; Theodore Cooke. Flora of the Presidency of Bombay. New Connaught place, Dehradun: 1997. p. 562. Vol. 2.
6. Britto SJ, Soosairaj S, Natarajan D, Nagamurugan N, Ravipaul S. *Euphorbia fusiformis* Buch.-Ham. (Euphorbiaceae): A new record for Tamil Nadu. J Econ Taxonomic Botany. 2002, 26:467–71.
7. Shah GC. Flora of Gujarat State. Vallabh Vidyanagar: Sardar Patel University; 1978. p. 621. Part 2.
8. Prakash A, Singh KK. Use of medicinal plants by certain tribal people in North India. J Trop Med Plants. 2001, 2:225–9.
9. Raju K, Natarajan S, Karuppusamy S, Manian S. New ethnobotanical report on *Euphorbia fusiformis* from Chitteri Hills of Tamil Nadu, India. J Swamy Botani Club. 2004;21:79–80.
10. Prakash, D., Upadhyay, G., Sing, B.N., Sing, H.B., Antioxidant and free radical Scavenging activities of seeds and agri-wast of some varieties of soybean. *Food Chem*., 2007, 104: 783-790.
11. Etsuo, N.,. Assessment of antioxidant capacity *in vitro* and *in vivo*. *Free Radic. Biol. Med*., 2010, 49: 503-515.
12. Halliwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I., Free radicals and antioxidants in food and *in vivo*: What they do and how they work. *Critical Reviews in Food Science and Nutrition*, 1995. 35: 7-20.

13. Simon, H.U., Haj-Yehia, A. and Levi-Schaffer, F., Role of reactive oxygen species (ROS) in the apoptosis induction. *Apoptosis*, 2000.5: 415-418.
14. Klein, S.M., Cohen, G. and Cederbaum, A.I., Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry*, 1991, 20: 6006-6012.
15. Hochestein, P. and Atallah, A.S., The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. *Mutation Research*, 1988. 202: 363-375.
16. Singh, R.P., Chidambara Murthy, K.N. and Jayaprakasha, G.K., Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *Journal of Agricultural and Food Chemistry*, 2002.50: 81-86.
17. Kotamballi, N., Murthy, K.N.C., Singh, R.P. and Jayaprakasha, G.K., Antioxidant activities of grape (*Vitis vinicifera*) pomace extracts. *Journal of Agricultural and Food Chemistry*, 2002.50: 5909-5914.
18. Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W. and Riechel, T.L., High molecular weight plant polyphenolics (Tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, 1998.46: 1887-1892.

**Table 1.Hydroxyl radical scavenging activity of different solvent extracts of *E. fusiformis* leaf.**

| Solvent | Concentration (µg) | Percentage activity (%) |
|---|---|---|
| **Chloroform** | 60 | 9.11 ± 0.04 |
| | 120 | 19.54 ± 1.03 |
| | 180 | 25.67 ± 3.10 |
| | 240 | 33.97 ± 0.73 |
| | 300 | 41.93 ± 0.42 |
| **Acetone** | 60 | 11.62 ± 0.31 |
| | 120 | 27.86 ± 0.54 |
| | 180 | 42.73 ± 1.21 |
| | 240 | 57.91 ± 3.12 |
| | 300 | 67.55 ± 0.81 |
| **Ethanol** | 60 | 40.42 ± 0.53 |
| | 120 | 47.16 ± 0.30 |
| | 180 | 58.68 ± 0.30 |
| | 240 | 69.14 ± 0.53 |
| | 300 | 84.75 ± 1.70 |
| **Water** | 60 | 20.29 ± 0.01 |
| | 120 | 31.57 ± 1.24 |
| | 180 | 46.83 ± 2.54 |
| | 240 | 51.76 ± 1.20 |
| | 300 | 60.54 ± 0.13 |

Values are means of three independent analyses ± standard deviation (n = 3).

**Table 2. Hydroxyl radical scavenging activity of different solvent extracts of *E. fusiformis* rhizome.**

| Solvent | Concentration (µg) | Percentage activity (%) |
|---|---|---|
| **Chloroform** | 60 | 12.36 ± 0.37 |
| | 120 | 20.22 ± 0.37 |
| | 180 | 28.96 ± 0.43 |
| | 240 | 36.45 ± 0.57 |
| | 300 | 48.06 ± 0.57 |
| **Acetone** | 60 | 3.18 ± 0.29 |
| | 120 | 6.36 ± 0.29 |
| | 180 | 15.51 ± 0.60 |
| | 240 | 20.23 ± 0.58 |
| | 300 | 25.53 ± 0.44 |
| **Ethanol** | 60 | 24.41 ± 0.51 |
| | 120 | 34.11 ± 0.29 |
| | 180 | 45.88 ± 0.78 |
| | 240 | 52.16 ± 0.45 |
| | 300 | 62.84 ± 0.61 |
| **Water** | 60 | 7.14 ± 0.27 |
| | 120 | 12.73 ± 0.16 |
| | 180 | 19.60 ± 0.42 |
| | 240 | 24.27 ± 0.57 |
| | 300 | 29.76 ± 0.42 |

Values are means of three independent analyses ± standard deviation (n = 3).

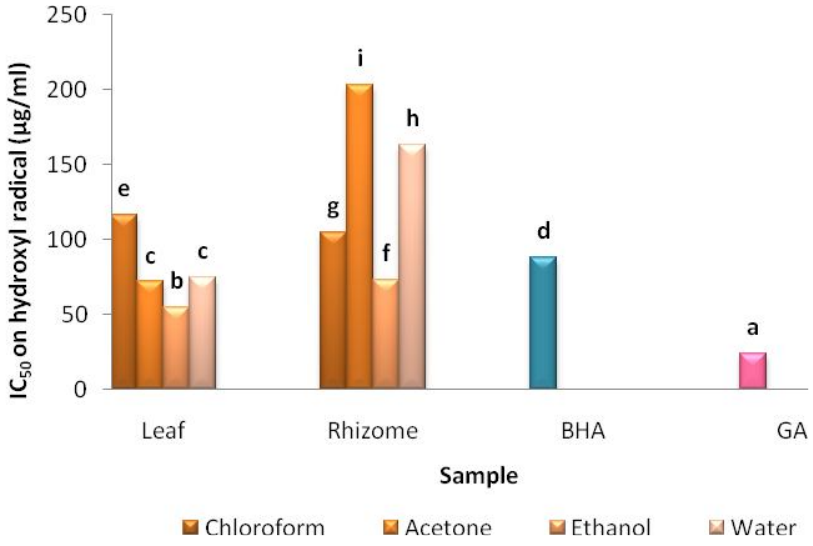


**Fig.1. $IC_{50}$ of different solvent extracts of *Euphorbia fusiformis* leaf and rhizome on hydroxyl radical**
Mean values statistical difference (P<0.05). BHA – Butylated hydroxyanisole; GA – Gallic acid.

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REVIEW ARTICLE

# Anticancer Drugs from Medicinal Plants-an Over view.

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**ABSTRACT**

India is the largest producer of medicinal plants and is rightly called "Botanical Garden of the world". Herbal medicines have a vital role in the prevention and treatment of cancer. Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines. Medicinal herbs have been in the fore front whenever we talk about anticancer remedies. A large number of herbal medicines are used by cancer patients for treating the cancer. Various types of anticancer plant are *Astragalus, Azadirachta indica, Betula alba, Brassica oleracea, Catharanthus roseus, Colchicum autumnale, Curcuma longa, Viscum album*.L, and others. There is a broad scope to derive the potent of anticancer agents from medicinal plants which need thorough research. Further studies are warranted to investigate potentially harmful herbal interactions with anticancer drugs in patients.

**Keywords:** Anticancer, Medicinal Plants, Cancer Treatment, Phytoconstituents derived from the Plants.

## INTRODUCTION

Over the past decades, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Because of their natural origin and less side effects. Many traditional medicines use are derived from medicinal plants, minerals and organic matter.The World Health Organisation(WHO) has listed 21,000 plants which are used for medicinal purposes around the world. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals and health care. Herbal medicines have a vital role in the prevention and treatment of cancer which probably the most important genetic disease. Medicinal herbs are commonly available and comparatively economical. A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany,

France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer. Some herbs reduce toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. Despite the long history of cancer treatment using herbal remedies in the province, the knowledge and experience of these herbalists have not been scientifically documented. The cancer review focuses on herbal drug preparation and plants used in the treatment of cancer.

## Cancer and its Causes

Cancer is a general term applied of series of malignant disease that may affect different parts of body. It is a group of diseases caused by loss of cells cycle control. If the process is not arrested, it may progress until; it causes the death of the organism. Cancer cells may invade nearby tissues and they may spread through the blood stream and lymphatic system to other parts of the body. The process by which a normal all changes in to one that behaves so abnormally can take a long time and is often triggered by outside influences. Despite the difference between different types of cancer, all cancers share some common features, and these shared properties are the basis for many cancer treatments and research efforts.

Primarily there are various genetic changes that occur inside the cells causing them to divide in an uncontrolled manner. Cancer begins with damage in DNA. DNA is like a set of instructions for cells, telling them how to grow and divide. The ultimate cause, regardless of genetic property or viruses that may influence the risk of the cancer, is often exposure to carcinogenic chemicals and / or to radiation, coupled with a failure of the immune system to eliminate the cancer cells at an early stage in their multiplication. Other factors such as smoking, alcohol consumption, excess use of caffeine and drugs, sunshine, diet as well as chemicals and radiation in our homes and workplaces.

## Types of Cancer disease

Blood Cancer, Bone Cancer, Brain Cancer, Breast Cancer, Endocrine Cancer, Eye Cancer, Genitourinary Cancer Gynecologic Cancer, Head and Neck Cancer, Respiratory Cancer and Skin Cancer

## The Mechanism on Cancer Therapy

- Inhibiting cancer cell proliferation directly by stimulating macrophage phagocytises, enhancing natural killer cell activity.
- Promoting apoptosis of cancer cells by increasing production of interferon, interleukin-2 immunoglobulin and complement in blood serum.
- Enforcing the necrosis of tumour and inhibiting its translocation and spread by blocking the blood source of tumour tissue.
- Enhancing the number of leukocytes and platelets by stimulating the hemopoietic function.
- Promoting the reverse transformation from tumour cells into normal cells.
- Promoting metabolism and preventing carcinogenesis of normal cells.
- Stimulating appetite, improving quality of sleep, relieving pain, thus benefiting patient's health.

## Natural Products

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries. The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Medicinal plants represent a vast potential resource for anticancer compounds. As with all areas of phytomedicine, the value of medicinal plants lies in the potential access to extremely complex molecular structures that would be difficult to synthesize in the laboratory.

### *Astragalus propinquus*

*Astragalus* is the large genus of about three hundred species. The genus is native to temperate regions of the Northern Hemispheres.The natural gum tragacanth is made from several species of *astragalus*. *Astragalus propinquus* has a history of use as an herbal medicine used in systems of traditional Chinese medicine. The National Institute of Health states" the evidence for using *astragalus* for any health condition is limited..*astragalus,* either alone or in combination with other herbs, may have potential benefits for the immune system, heart and liver and as an adjunctive therapy for the cancer. There are mixed data regarding *astragalus,* its effect on telomerase and cancer. Scientists propose that "forced" elongation of telomeres promotes the differentiation of cancer cells, probably reducing malignancy, which is strongly associated with a loss of cell differentiation. *Astragalus* can be used not only as a general tonic and immune system booster in health uses. But a means to health cancer sufferers (Breast, Cervix and Lung types of cancer were treated during the studies) overcome their disease used in combination with radiation and chemotherapy.The studies in mice concluded that *astragalus* works by inhibiting the growth of the tumors.

### *Azadirachta indica*

*Azadirachta indica* is known as Neem tree, a member of the meliaceae family. It is one of two species in the genus azadirachta indica and is native to India, Pakistan and Bangladesh. Multi directional therapeutic uses of Neem have been known in India since the *Vedic* times. All parts of the tree have been in use as traditional medicine for human hold remedies against various ailments from antiquity. Over to different types of biochemicals including terpinoids and steroids have been purified from this plant. Preclinical research work done during the last decade has fine tuned our understanding of the anticancer properties of the crude and purified products. From this plant the anticancer properties of the plant have been studied largely in terms of its preventive, protective, tumor suppressive immunomodulatives and apoptotic effects against various types of cancer and their molecular mechanisms. Two recent reports suggest that neem pretreatment also enhances activity by reducing the side effects of some conventional cancer treatments.

### *Betula alba*

White birch bark (*Betula alba*) which contains betulinic acid, has been used by Native Americans as a folk remedy. *Betula pendula* (silver birch) is a species of tree in the family Betulaceae. The outer part of the bark contains up to 20% betulin.Betulinic acid is a pentacyclic triterpenoid of plant origin that is widely distributed in the plant kingdom throughout the world. Betulinic acid has been shown to have antitumor properties. Betulinic acid has also been reported to inhibit aminopeptidase N, an enzyme that is involved in the regulation of angiogenesis and overexpressed in several cancers Alterations in cell cycle progression in response to betulinic acid were also highly dependent on individual cell lines. Whether betulinic acid-mediated cell cycle changes are linked to its antitumor activity remains to be addressed in future studies. While initial reports suggested that betulinic acid is selectively cytotoxic against melanoma cell lines, anticancer activity was subsequently also reported against other

### *Brassica oleracea*

*B. oleracea* has become established as an important human food crop plant, used because of its large food reserves, which are stored over the winter in its leaves. It is rich in essential nutrients including vitamin C Cruciferous vegetables, including broccoli, are being investigated for a potential role in the prevention and treatment of cancer. Multiple mechanisms of action for broccoli and its constituents have been proposed based largely on cell culture studies. Published reviews of epidemiological data investigating the role of broccoli in cancer prevention have not all demonstrated a positive protective effect, but harm was not demonstrated. No association was found for flavonoid - rich foods or broccoli with the incidence of total and site-specific cancers among middle-aged and older women in

the Women's Health Study. Likewise, no association was demonstrated for flavonoids and the incidence of ovarian cancer; however, an inverse association with broccoli intake, attributed to kaempferol, was found. In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, vegetable and fruit consumption was not related to prostate cancer risk overall, but broccoli appeared to be protective for risk of aggressive, extraprostatic prostate cancer. The European Prospective Investigation into Cancer and Nutrition study found no association between cruciferous vegetable consumption and the risk of prostate cancer. In the Health Professionals Follow-Up Study, 5 or more servings of broccoli per week showed a protective effect for bladder cancer, while 2 further reviews showed 3 to 5 servings per week to be protective for prostate cancer. A review of epidemiological data for prostate cancer found modest support for a protective effect of Brassica vegetables, with 4 of 12 studies demonstrating statistically significant protection but noting bias to be a particular problem. The incidence of Barrett esophageal adenocarcinoma (BEAC) has been increasing at an alarming rate in western countries. That is a natural product with antioxidant properties from broccoli has great potential to be used in chemoprevention and treatment of BEAC.

### *Catharanthus roseus*

In Ayurveda (Indian traditional medicine) and Chinese medicine the extracts of its roots and shoots, though poisonous, is used against several diseases. In traditional, including diabetes, malaria, and Hodgkin's lymphoma. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin's lymphoma. The vinca alkaloids are a subset of drugs derived from the Madagascar periwinkle plant. They were discovered in the 1950's by Canadian scientists, Robert Noble and Charles Beer. Vinca alkaloids have been used to treat diabetes, high blood pressure, and the drugs have even been used as disinfectants. However, the vinca alkaloids are most famous for being cancer fighters. There are four major vinca alkaloids in clinical use: vinblastine, vinorelbine, vincristine, and vindesine. The vinca alkaloids are cytotoxics – they halt the division of cells and cause cell death.vinca alkaloids prevent cancer cells from successfully dividing. In 2005, cytotoxics represented the majority of the Top 20 Cancer Therapeutics, according to datamonitor.com. This was due to cytotoxics' long history in oncology and their key roles in current treatment. As of 2006, however, another type of drug has started to overtake the market. That drug type is called a molecular targeted therapy. The molecular targeted therapy class has been most responsible for overall growth experienced by the oncology market between 2005 and 2008. One reason for this is that molecular targeted therapies lack the generic competition endured by cytotoxics like the vinca alkaloids. Overall, vinca alkaloids are in the second most-used class of cancer drugs but sales are expected to decline. Nonetheless, vinca alkaloids will remain among the fundamental cancer therapies.

### *Colchicum autumnale*

*Colchicum autumnale*, commonly known as autumn crocus, meadow saffron or naked lady, is a flower that resembles the true crocuses, but blooms in autumn. The bulb-like corms of *Colchicum autumnale* contain colchicine, a useful drug with a narrow therapeutic index. In the early stages of drug development for the treatment of some types of cancer. In experimental testing it was successfully used to treat breast, bowel, lung and prostate cancers in mice when used in combination with the drug doxorubicin. The utility of colchicine for cancer therapy is currently limited, as only doses close to the maximal tolerated dose (MTD) can induce reduction in tumor blood perfusion leading to a high risk for toxicity Colchicine shows antimitotic activity and used in cancer for the dispersal of tumours and for treatment of various neoplastic diseases.

### *Curcuma longa*

Curcumin (diferuloylmethane) is a polyphenol derived from the *Curcuma longa* plant, commonly known as turmeric. Curcumin has been used extensively in Ayurvedic medicine for centuries, as it is nontoxic and has a variety of therapeutic properties including anti-oxidant, analgesic, anti-inflammatory and antiseptic activity. More recently curcumin has been found to possess anti-cancer activities via its effect on a variety of biological pathways involved in

mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis. Curcumin has shown anti-proliferative effect in multiple cancers. Its therapeutic activity in head and neck cancer as well as some of the challenges concerning its development as an adjuvant chemotherapeutic agent. Because of the far-reaching effects and multiple targets of curcumin on the cell growth regulatory processes, it holds much promise as a potential chemotherapeutic agent for many human cancers. In addition to the inhibitory effects on the production of nitric oxide (NO) and the ability to scavenge DNA-damaging superoxide radicals, curcumin also affects both the Phase I and Phase II enzymes of the hepatic cytochrome p450 enzyme system involved in the oxidation and detoxification of toxic substances. Conversely, curcumin induces the Phase II enzymes involved in detoxification of toxic metabolites (including glutathione S-transferase, glutathione peroxidase and glutathione reductase) . Curcumin's inhibitory effect on carcinogenesis has been demonstrated in several animal models of various tumor types including oral cancer, mammary carcinoma and intestinal tumors.

### *Viscum album*

*Viscum album* is a species of mistletoe in the family Santalaceae, commonly known as European mistletoe. The activity principle of the mistletoe (*Viscum album* L.) phytotherapeutics could be considered as combined cytotoxic and "biological response modifying" activities (increasing host defense against cancer) that result from the activities of the plant lectins and the other biologically relevant substances. Researchers found before that the aqueous extract Isorel, produced by Novipharm (Pörtschach, Austria) from the entire plant of fresh mistletoe under standardized conditions with bioassay validated batch consistency, can be valuable in experimental adjuvant cancer therapy increasing efficiency of cyclophosphamide chemotherapy.

## DISCUSSION

Science has long acknowledged the value of healing substances found in nature, such as digitalis, aspirin, penicillin, insulin, steroids, etc. There has been a resurgence of interest, both scientifically and popularly, in the utilization of natural approaches. Experiments on cell lines and in animals demonstrated that herbal drugs anticancer role by inducing apoptosis and differentiation. However, the mechanism of the anticancer role has not yet been fully elucidated. Further research is needed to explore the molecular mechanism of herbal drugs. Although the clinical trials showed that herbs were helpful against cancer. Though many of them demonstrated that herbs are helpful against cancer, especially useful in improving survival and quality of life in patients suffering from advanced cancer, the lack of controls and reporting bias have been severe flaws. Researchers must pay attention to the scientific rigor of studies of herbal drugs in the future to improve the status. In future research is required to determine which ingredients are effective which will provide valuable clues for researching and developing anticancer drugs in the future.

## REFERENCES

1. Grover J.K., Yadav S., Vats V. Medicinal plants of India with antidiabetic potential. J.Ethnopharmacol. 2002;81:81–100.
2. Seth S.D., Sharma B. Medicinal plants of India. Indian J. Med. Res. 2004;120:9–11.
3. Larkin T. Herbs are often more toxic than magical. FDA Consum 1983;17:4–11.
4. Saxe TG. Toxicity of medicinal herbal preparations. Am Fam Physician 1987;35:135–42.
5. Tyler V. Herbs of choice. The therapeutic use of phytomedicinals. New York: Haworth Press, 1994, 24-26.
6. McNutt K. Medicinals in foods. Nutr Today 1995;30:218–22.
7. Cancer index web site. Available at: http/www.cancerindex.org
8. http/www.cancer.gov.
9. Savita Dixit and Huma Ali .Anticancer Activity of Medicinal plant extract-A Review.j.chem.& Cheml.Sci.vol.1(1),79-85 (2010).

10. Sakarkar D.M., Deshmukh V.N.Sudhakarrao Naik . Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity. International Journal of PharmTech Research. Vol. 3, No.1, pp 298-308.
11. Zargary, A. Medicinal plants. 5th Edition.Tehran: Tehran University Publications 1990; pp. 312-314.
12. Paul R, Prasad M, Sah NK Anticancer biology of Azadirachta indica L (neem): a mini review.Cancer Biol Ther. 2011 Sep 15;12(6):467-76. doi: 10.4161/cbt.12.6.16850. Epub 2011 Sep 15.
13. S. Zillur Rahman and M. Shamim Jairajpuri. *Neem in Unani Medicine*. Neem Research and Development Society of Pesticide Science, India, New Delhi, February 1993, p. 208-219. Edited by N.S. Randhawa and B.S. Parmar. 2nd revised edition (chapter 21), 1996
14. Betulinic Acid for Cancer Treatment and Prevention Int J Mol Sci. 2008 June; 9(6): 1096–1107.
15. Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR, Cordell GA, Beecher CW, Fong HH, Kinghorn AD, Brown DM. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat Med. 1995 Oct; 1(10):1046-51.
16. Hunt, D., ed. (1993). Betula. Proceedings of the IDS Betula Symposium 2–4 October 1992. International Dendrology Society .
17. Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, van den Brandt PA (1996)Epidemiological studies on brassica vegetables and cancer risk.Cancer Epidemiol Biomarkers Prev5(9):733–748.
18. Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. Chemical and biological characterisation of nutraceutical compounds of broccoli. J Pharm Biomed Anal . 2006;41(5):1508-1522.
19. Finley JW. Reduction of cancer risk by consumption of selenium-enriched plants: enrichment of broccoli with selenium increases the anticarcinogenic properties of broccoli. J Med Food . 2003;6(1):19-26.
20. Fimognari C, Lenzi M, Hrelia P. Interaction of the isothiocyanate sulforaphane with drug disposition and metabolism: pharmacological and toxicological implications. Curr Drug Metab . 2008;9(7):668-678.
21. Safe S, Papineni S, Chintharlapalli S. Cancer chemotherapy with indole-3-carbinol, bis(3′-indolyl)methane and synthetic analogs. Cancer Lett . 2008;269(2):326-338.
22. Aggarwal BB, Ichikawa H. Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. Cell Cycle . 2005;4(9):1201-1215.
23. Jeffery EH, Keck AS. Translating knowledge generated by epidemiological and in vitro studies into dietary cancer prevention. Mol Nutr Food Res . 2008;52(suppl 1):S7-S17.
24. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett . 2008;269(2):291-304.
25. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. Cell Mol Life Sci . 2007;64(9):1105-1127.
26. Myzak MC, Dashwood RH. Chemoprotection by sulforaphane: keep one eye beyond Keap1. Cancer Lett . 2006;233(2):208-218.
27. Lampe JW, Peterson S. Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr . 2002;132(10):2991-2994.
28. Beecher CW. Cancer preventive properties of varieties of Brassica oleracea : a review. Am J Clin Nutr . 1994;59(5)(suppl):1166S-1170S.
29. Minich DM, Bland JS. A review of the clinical efficacy and safety of cruciferous vegetable phytochemicals. Nutr Rev . 2007;65(6, pt 1):259-267.
30. Rogan EG. The natural chemopreventive compound indole-3-carbinol: State of the science. In Vivo . 2006;20(2):221-228.
31. Harv Womens Health Watch. 2005;12(6):8.
32. Jean Bruneton, Pharmacognosy, phytochemisty medicinal plants, Lavoisier Publisher, France,1993 pp. 832.
33. Wang J, Ito H, Shimura K. Enhancing effect of antitumor polysaccharide from *Astralagus* or *Radix hedysarum* onC3 cleavage production of macrophages in mice. Department of Pharmacology, Mie University School of Medicine, Japan. MemInst Oswaldo Cruz; 86 2: 159-164,1991.20
34. E.Balasubramanian and T.Gajendran.Comparative studies on the anticancer activity of colchicine by various controlled drug delivery modes.int j pharm bio sci2013 jan;4(1):(p)9-26.

35. Yang AK, He SM, Liu L, Liu JP, Wei MQ, Zhou SF. Herbal interactions with anticancer drugs: mechanistic and clinical considerations. Curr Med Chem. 2010;17(16):1635-78.
36. Reason Wilken, Mysore S Veena, Marilene B Wang, and Eri S Srivatsan Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma
37. Thapliyal R, Maru GB. Inhibition of cytochrome p450 isoenzymes by curcumins in vitro and in vivo. Food Chem Toxicol. 2001;39:541–547.
38. Krishnaswamy K, Goud VK, Sesikeran B, Mukundan MA, Krishna TP. Retardation of experimental tumorigenesis and reduction in DNA adducts by turmeric and curcumin. Nutr Cancer. 1998;30:163–166.
39. Inano H, Onoda M, Inafuku N, Kubota M, Kamada Y, Osawa T, Kobayashi H, Wakabayashi K. Chemoprevention by curcumin during the promotion stage of tumorigenesis of mammary gland in rats irradiated with gamma-rays. Carcinogenesis. 1999;20:1011–1018. doi: 10.1093/carcin/20.6.1011.
40. Collett GP, Robson CN, Mathers JC, Campbell FC. Curcumin modifies Apc(min) apoptosis resistance and inhibits 2-amino 1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induced tumour formation in Apc(min) mice.
41. Carcinogenesis. 2001;22:821–825. doi: 10.1093/carcin/22.5.821.
42. Zarkovic N, Vukovic T, Loncaric I, Miletic M, Zarkovic K, Borovic S, Cipak A, Sabolovic S, Konitzer M, Mang S. An overview on anticancer activities of the Viscum album extract Isorel. Cancer Biother Radiopharm. 2001 Feb;16(1):55-62.
43. Böhling, N., Greuter, W., Raus, T., Snogerup, B., Snogerup, S. & Zuber, D. (2003). Notes on the Cretan mistletoe, Viscum album subsp. creticum subsp. nova (Loranthaceae/Viscaceae). *Israel J. Pl. Sci*. 50 (Suppl.): 77-84.

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RESEARCH ARTICLE

# Ethnopharmacological Investigation of *Vitex altissima* L.f.(Verbenaceae).

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**ABSTRACT**

This paper evaluated ethnopharmacological investigation of *Vitex altissima* L.f. (verbenaceae) at Servaroyan hills (yercaud) of Eastern ghats, salem district,Tamil nadu. The information obtained can lead to directions of discovery of new drugs. On *invitro* studies *V.altissima* the air dried, powdered leaf, stem bark, fruit was extracted successively in soxhlet extractor with acetone, methanol and water. Phytochemical analysis of alkaloids, flavanoids, tannins, saponins, phenols, carbohydrates, proteins and glycosides are examined. DPPH radical scavenging activity was evaluated, and acute toxicity was observed.

**Keywords:**Ethnopharmacological, *Vitex altissima* L.f. , verbenaceae , phytochemical, antioxidant, acute toxicity.

## INTRODUCTION

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances [1-4].No one knows exactly how many different medicinal plants are used in the world today, analyzing the phytochemicals in medicinal plants provides scientists with insight into how effective plants are medicinally, and understanding how and why they are effective can lead to the development of new medicines [5].

Phytochemical analysis of medicinal plants has shown that numerous compounds in plants traditionally used for medicinal purposes have chemical properties effective at treating illness. Free radicals induce oxidative stress *in-vivo* that may lead to oxidative modification or damage of some biological structures such as lipids, proteins, DNA and may give rise to degenerative diseases. There is need for antioxidant intervention which one of the plants studied may be of importance [6].Early pharmacologists focused on natural substances, mainly plant extracts. Pharmacology developed in the 19th century as a biomedical science that applied the principles of scientific experimentation to therapeutic context [7-10].The toxicity study which is essential for an adaption of the traditional medicine was conducted to identify the tolerance limits of plant extracts [5]. So that, the present study was conducted on *V.altissima* to analyses the phytochemical constituents, antioxidant capacities of the plant extracts was determined in terms of radical scavenging ability, by using the stable radical DPPH and evaluate their acute toxicity in mice.

## MATERIALS AND METHODS

**Plant description**

The genus *Vitex* contains 270 species all over the world with diverse medicinal active constituents and properties[11].The present work was carried out on *Vitex altissima*. Family: Verbenaceae Common name: peacock chaste tree. A large tree grows up to 40 meters in height. Leaves compound, palmate, 3-5 foliate, winged rachis, leaflets acute, ovate, membranous, and glabrous. Flowers whitish, arises from terminal panicles. Fruits ovoid drupes containing hard seed.

***In vitro* studies**

**Solvent extraction**

The air dried, powdered leaves, stem bark and fruit of *Vitex altissima* was extracted successively in soxhlet extractor with acetone, methanol and water. each time, before extracting with the next solvent, the powdered material was dried in hot air oven at 40°c.All the three different solvent extracts were evaporated to remove the final traces of the respective solvents. The percentage yield was calculated. The extracts were subjected to the following *in vitro* assays.

**Qualitative analysis**

Phytochemical screening of different solvent extracts was carried out following the methods of Horbone, 1984[12], Kokate *et al*., 1995 [13] and Prabhakaran, 1996[14].

**Test for alkaloids**
**Wagner's test**

To 1 ml of the extract, a few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

**Test for Flavonoids**
**Shinoda Test**

To 1ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink color indicates the presence of Flavonoids.

**Test for Tannins**

To 1ml of the extract, few ml of lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

**Test for steroids and sterols**
**Salkowski's test**

The extract was dissolved in 2ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extract.

**Test for Carbohydrates**
**Fehling's test**

Five ml of Fehling's solution was added to 2 ml of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

**Test for Saponins**

5 ml of the extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes. Formation of honey comb like froth shows the presence of saponins.

**Test for Phenols**

To 5 ml of extract, 1 ml of 1% lead acetate solution was added. Formation of yellow precipitate shows the presence of phenols.
**Test for Glycosides**

A small amount of filtrate was dissolved in 1ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

**Test for Protein**
**Biuret test**

To 1ml of extract, equal volume of 40% NaOH solution and two drops of 1% Copper sulphate solution were added. The appearance of violet colour indicates the presence of protein.

**Free radical scavenging activity on DPPH**

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois(1958)[15].The sample extracts at various concentrations (20 - 100 µg) was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517nm. BHA and BHT were used as positive control. Percentage radical scavenging activity of the sample was calculated as follows:

$$\% \text{ DPPH radical scavenging activity} = (\text{control OD-sample OD} / \text{control OD}) \times 100$$

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

***In vivo* studies**
**Solvent extraction**

The air dried, powdered stem bark of *Vitex altissima* was extracted in soxhlet extractor with ethanol. The extract was evaporated to remove the final traces of ethanol. The percentage yield was calculated. The extracts were subjected to the following *in vivo* studies.

**Experimental animals**

Animals used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark /10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted over night before the experimental schedule, but have free access for water *ad libitum.* The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethic committee.

**Acute toxicity**

Acute oral toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines (Ecobichon, 1997)[16].Swiss albino male mice (n = 6/each dose) selected by random sampling technique were employed in this study. The animals were fasted for 12 h with free access to water only. Ethanol extract of *Vitex altissima* (dissolved in distilled water) were administered orally at a dose of 5 mg/kg initially to mice and mortality was observed for 3 days. If mortality was observed in 4/6–6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of six animals then the same dose was repeated with higher doses such as 1000, 2500, 5000 and 10000 mg/kg. The general behaviors such as mortality and clinical signs, which includes changes in skin fur, eyes and mucous membranes, were observed for the first 1 h and after 24 h of test drug administration. The gross behaviors like body positions, locomotion, rearing, tremors and gait was observed. The effect of plant extract on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, body weight and intake were also observed.

## RESULTS AND DISCUSSION

Phytochemicals are plant derived chemical compounds. This experiment was carried out to find the presence or absence of alkaloids, flavonoids, saponins, phenols, tannins, carbohydrates, proteins and glycosides. For this experiment plant extracts were taken by using the solvent of acetone, methonal and water. The results showed that leaf, stem bark and fruit extracts of *V.altissima* have the presence of the tested active principle in all the three extracts, except saponins are absent in the leaf, stem bark and fruit of acetone extracts, at the same carbohydrates absent in methanol and water extracts of leaf and in stem bark also carbohydrates absent in all the three extracts. The protein and amino acids are absent in leaf and stem bark of acetone extracts. (Table.1).The phytochemical study offers a base of using *V.altissima* as herbal alternative [17]. Phenol, flavonoids and tannins are good antioxidant substances which prevent or control oxidative stress related disorders [18,19].Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, aging, etc. in the treatment of these diseases, antioxidant therapy has gained utmost importance[8,9].The antioxidant activity of the extracts was determined in terms of radical scavenging ability, using the stable radical DPPH. From this studies leaf, stem bark, and fruit extracts is taken by using the

solvent acetone, methanol and water, the sample extracts at various concentration providing 50% inhibition ($IC_{50}$) under the assay condition was calculated. From this experiment results *V.altissima,* acetone extracts of leaf shows more IC50 values when compare to other two extracts, (Table.2). As in the stem bark of *V.altissima* water extracts has more $IC_{50}$ values (Table.3) and in fruits acetone extracts has high $IC_{50}$ values (Table.4).Compare to standard BHA and GA, DPPH radical scavenging activity in *V.altissima* have more $IC_{50}$ values.

**Acute toxicity**

Acute oral toxicity studies were performed on Swiss albino male mice (n = 6/each dose) and they were selected by random sampling technique. Ethanol extracts of *V.altissima* was administered orally at a dose of 5mg/kg initially and then the same does was repeated with higher doses such as 1000, 2500, 5000 and 10,000 mg/kg. The general behaviors such as mortality and clinical signs, which includes changes in skin fur, eyes, and mucous membranes, were observed for the first 1hr and after 24hr of test drug administration. From this observation results showed all animals are survived and appeared active and healthy and no significant changes in the general behavior. The same genus Vitex was reported by Ramasamy Anandan et al., (2009) have reported *V. trifolia* plant extracts did not show toxicity effects [20] and cancer biomass database had also reported *V.leucoxylon* plant extract had no toxic effects [21].

## CONCLUSION

In this study were conducted to understand the uses of plants, to identify their phytochemical composition, DPPH radical scavenging ability and to evaluate their acute toxicity in mice. Based on the experimental results we concluded *V.altissima* had qualitative phytochemical composition such as alkaloids, flavonoids, saponins, phenols, steroids, tannins, carbohydrates, proteins, amino acid and glycosides. In the DPPH radical scavenging activity is observed in leaf, stem bark, and fruit extracts. On the observation compare with other parts of *V.altissima* the water solvent of stem bark showed high scavenging values against the DPPH radical. These results clearly indicate that *V.altissima* is effective against free radical mediated diseases. For the acute oral toxicity studies the plant extracts did not produce any toxic effect to the animal or no mortality up to 10,000 mg/kg body weight. We finally concluded the plant extracts can be considered to have fairly high margin of safety.

## REFERENCES

1. Mohan.V.R.,P.Chenthurpandy and C.Kalidass, 2010. Pharmcognostic and phytochemical investigation of *Elephantopus scaber* L. (asteraceae). Journal of pharmaceutical science and technology, vol. 2(3): 191-197.
2. Marikani Kannan and A.J.A. Ranjit Singh, 2010. An Immuno–Pharmacological Investigation of Indian Medicinal plant *Nyctanthes arbor-tristis* Linn. World Applied Sciences Journal, 11(5): 495-503.
3. Nayak B.S. and K.N.Patel, 2010. Pharmacognostic studies of the *Jatropha curcas* leaves, International Journal of Pharm Tech Research; vol.2,No.1, pp 140-143.
4. Shankar,D and Ved, D.K. 2003. Indian forester, 129: 275-288.
5. Wagnert H., 1999. New approaches in Phytopharmacological research.Pure Appl.Chem., Vol.71,No.9:1649-1654.
6. Rana M.G.., R.V.Katbamna., A.A .Padhya., A.D.Dudhrejiya., N.P.Jivant., N.R.Sheth, 2010. Invitro antioxidant and free radical scavenging studies of alcoholic extract of Medicago sativa L..Rom.J.Biol.Plant Bio L., Volume 55,No 1: 15-22.
7. Mannfred A.Hellinger, 2003. Introduction to pharmacology. CRC Press. P. 4. ISBN 0-415-28033-8.
8. Rang HP,2006. The receptor concept: Pharmacologys big idea. Br.J.Pharmacol.147 Suppl 1 (S1):S9-16.
9. Rang H.P., M.M.Dale., J.M.Ritter.,R.J.Flower, 2007. Pharmacology china :Elsevier, ISBN 0-443-06911-5.
10. Kabore Adama, Tamboura Hamidou H., Traore Aristide, Troare Amadou, Meda Roland, Kiendrebeogo Martin, Belem A.M.Gaston,Sawadoga Laya ,2010.Phytochemical analysis and acute toxicity of two medicinal plants

(*Anogeissus leiocarpus* and *Daniellia oliveri*) used in traditional veterinary medicine in Burkina Faso.Scholars Research Library. Archives of Applied Science Research,2(6):47-52.

11. Kulkarni L.A, 2011.Pharmacological Review on *Vitex trifolia* Linn (Verbenaceae). Pharmacology online 3:858-863.
12. Horbone, J.B.,1984. In: Phytochemical methods, 2nd edition.Chapman and Hall, Newyork.
13. Kokate, C.K., Purohit, A.P and Gokhale, S.B., 1995. In: Pharmacognosy, 3rd edition. Niralin Prakashan, Pune.
14. Prabhakaran, P., 1996. Chemical investigation of finding medicinal plants and related synthetic studies. Ph.D thesis. M.K.U., Madurai, India.
15. Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, **26**: 1199-1200.
16. Ecobichon, D.J., 1997. The basis of toxicology testing. CRC Press, New York, pp.43–86.
17. Sahaya Sathis S.,Janakiraman N.,Johnson M.2012.Phytochemical analysis of *Vitex altissima* L.using UV-VIS,FTIR and GC- MS.Internation Journal of pharmaceutical sciences and drug research:4(1):56-62.
18. Vinson J A., Jang J., Dabbagh Y A., Serry M M., Cai S. 1995a. Plant phenols exhibit lipoprotein – bound antioxidant activity using an invitro model for heart disease. J Agric Food chem.43: 2798-2799.
19. Vinson J A., Jang J., Dabbagh Y A., Serry M M.,Jang J. 1995. Plant flavonoids, especially tea flavonoids, are powerful antioxidents using an invitro oxidation model for heart disease. J Agric Food chem.43: 2798-2799.
20. Ramasamy Anandan, Balasundaram jayakar, Biusan Kumar,Seevalen Babuji, Rajappan Manavalan and Raju Senthil Kumar ,2009.Effect of ethanol extract of flowers of *Vitex trifolia* Linn. On $CCl_4$ induced the hepatic injury in rats.Pak.J.Pharm.Sci., Vol.22,No.4:391-394.
21. Drug target screening assays. Cancer biomarker database with companion diagnostic pathway maps. Acute oral toxicity assay using up down procedure on rats. Source :7,780,997.

**Table 1.Qualitative phytochemical evaluation of different solvent extracts of *Vitex altissima***

| **Phyto chemical Test** | **Samples** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaf** | | | **Stem bark** | | | **Fruits** | | |
| **Alkaloids** | Acetone extract | Methanol extract | Water extract | Acetone extract | Methanol extract | Water extract | Acetone extract | Methanol extract | Water extract |
| **Flavonoids** | + | + | + | + | + | + | + | + | + |
| **Saponins** | + | + | + | + | + | + | + | + | + |
| **Phenols** | – | + | + | – | + | + | – | + | + |
| **Steroids** | + | + | + | + | + | + | + | + | + |
| **Tannins** | + | + | + | + | + | + | + | + | + |
| **Carbohydrates** | + | + | + | + | + | + | + | + | + |
| **Protein& Amino acids** | + | – | – | – | – | – | + | + | + |
| **Glycosides** | – | + | + | – | + | + | + | + | + |
| **Alkaloids** | + | + | + | + | + | + | + | + | + |
| **Flavonoids** | + | + | + | + | + | + | + | + | + |

'+' presence of compounds; '--' absence of compounds.

**Table 2.DPPH radical scavenging activity of *Vitex altissima* leaf.**

| Solvent | Sample concentration(µg) | Percentage activity | $IC_{50}$(µg/ml) |
|---|---|---|---|
| Acetone | 20 | 12.38±0.44 | 33.37±0.06$^{e}$ |
| | 40 | 23.31±0.93 | |
| | 60 | 35.74±0.94 | |
| | 80 | 47.11±0.93 | |
| | 100 | 61.02±0.50 | |
| Methanol | 20 | 12.70±0.47 | 33.05±0.19$^{e}$ |
| | 40 | 25.29±0.97 | |
| | 60 | 36.41±1.23 | |
| | 80 | 48.69±0.36 | |
| | 100 | 59.73±0.50 | |
| Water | 20 | 17.78±0.31 | 23.37±0.37$^{b}$ |
| | 40 | 34.73±0.89 | |
| | 60 | 52.02±0.94 | |
| | 80 | 68.44±0.82 | |
| | 100 | 84.86±2.06 | |

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P$<0.05).

**Table 3.DPPH radical scavenging activity of *Vitex altissima* stem bark.**

| Solvent | Sample concentration(µg) | Percentage activity | $IC_{50}$(µg/ml) |
|---|---|---|---|
| Acetone | 20 | 15.19±0.46 | 26.60±0.21$^{c}$ |
| | 40 | 29.85±0.50 | |
| | 60 | 45.63±0.96 | |
| | 80 | 60.46±0.60 | |
| | 100 | 74.71±0.80 | |
| Methanol | 20 | 16.29±0.81 | 24.68±0.07$^{b}$ |
| | 40 | 33.62±0.92 | |
| | 60 | 48.85±0.43 | |
| | 80 | 64.98±0.49 | |
| | 100 | 80.37±0.67 | |
| Water | 20 | 6.38±0.25 | 64.88±0.24$^{g}$ |
| | 40 | 12.60±0.25 | |
| | 60 | 18.69±0.18 | |
| | 80 | 24.25±0.12 | |
| | 100 | 30.51±0.14 | |

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P$<0.05).

**Table 4.DPPH radical scavenging activity of *Vitex altissima* fruit.**

| Solvent | Sample concentration(µg) | Percentage activity | $IC_{50}$(µg/ml) |
|---|---|---|---|
| **Acetone** | 20 | 5.66±0.08 | 87.72±0.17$^{h}$ |
| | 40 | 10.51±0.21 | |
| | 60 | 15.67±0.12 | |
| | 80 | 20.55±0.08 | |
| | 100 | 25.32±0.17 | |
| **Methanol** | 20 | 15.74±0.64 | 26.37±0.31$^{c}$ |
| | 40 | 29.48±1.04 | |
| | 60 | 46.77±1.13 | |
| | 80 | 60.00±0.81 | |
| | 100 | 78.87±1.15 | |
| **Water** | 20 | 8.64±0.25 | 49.54±0.36$^{f}$ |
| | 40 | 16.20±0.37 | |
| | 60 | 24.64±0.25 | |
| | 80 | 32.66±0.24 | |
| | 100 | 40.52±0.32 | |
| **BHA** | – | – | 5.20±0.26$^{a}$ |
| **GA** | – | – | 30.40±3.02$^{d}$ |

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P$<0.05). BHA – Butylated hydroxyanisole; GA – Gallic acid.

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**GENERAL ARTICLE**

# Sir Chandrasekhara Venkata Raman – Biographical.

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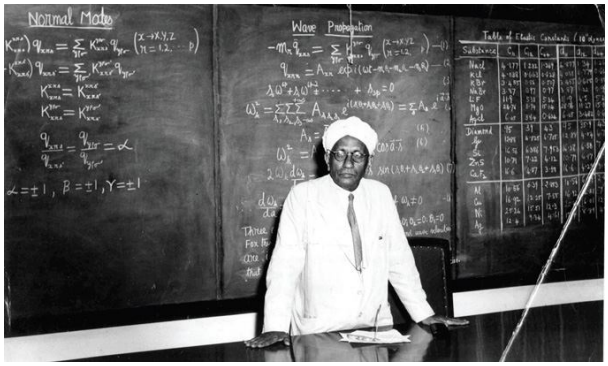
**BIOGRAPHY -1**

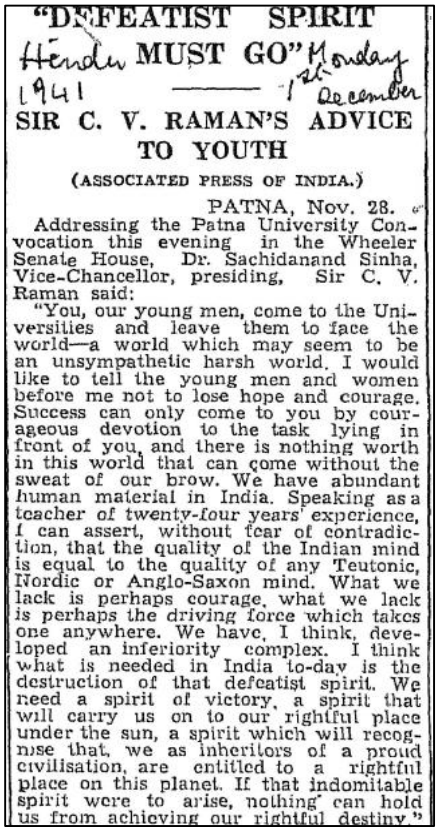
Sir C V Raman - Sir Chandrasekhara Venkata Raman, FRS (7 November 1888 – 21 November 1970) was an Indian physicist and Nobel laureate in physics recognised for his work on the molecular scattering of light and for the discovery of the Raman effect, which is named after him. Chandrashekhara Venkata Raman was born to a Hindu Brahmin family in Tiruchirapalli, Tamil Nadu. At an early age Raman moved to the city of Vizagapattinam, Andhra Pradesh. His father was a lecturer in mathematics and physics, so he grew up in an academic atmosphere.His nephew Subrahmanyan Chandrasekhar also won the Nobel Prize in Physics, in 1983.Raman entered medical College, Chennai, in 1902, and in 1904 gained his B.A., winning the first place and the gold medal in physics. In 1907 he gained his M.A., obtaining the highest distinctions. He joined the Indian Finance Department as an Assistant Accountant General in Kolkata. In 1917 Raman resigned from his government service and took up the newly created Palit Professorship in Physics at the University of Calcutta. At the same time, he continued doing research at the Indian Association for the Cultivation of Science, Calcutta, where he became the Honorary Secretary. Raman used to refer to this period as the golden era of his career. Many talented students gathered around him at the IACS and the University of Calcutta.

On February 28, 1928, through his experiments on the scattering of light, he discovered the Raman Effect. It was instantly clear that this discovery was an important one. It gave further proof of the quantum nature of light. Raman spectroscopy came to be based on this phenomenon, and Ernest Rutherford referred to it in his presidential address to the Royal Society in 1929. Raman was president of the 16th session of the Indian Science Congress in 1929. He was conferred a knighthood, and medals and honorary doctorates by various universities. Raman was confident of winning the Nobel Prize in Physics as well, and was disappointed when the Nobel Prize went to Richardson in 1928




and to de Broglie in 1929. He was so confident of winning the prize in 1930 that he booked tickets in July, even though the awards were to be announced in November, and would scan each day's newspaper for announcement of the prize, tossing it away if it did not carry the news. He did eventually win the 1930 Nobel Prize in Physics "for his work on the scattering of light and for the discovery of the effect named after him". He was the first Asian and first non-White to get any Nobel Prize in the sciences.Raman also worked on the acoustics of musical instruments. He worked out the theory of transverse vibration of bowed strings, on the basis of superposition velocities. He was also the first to investigate the harmonic nature of the sound of the Indian drums such as the tabala and the mridangam. In 1934 Raman became the director of the Indian Institute of Science in Bangalore, where two years later he continued as a professor of physics. Other investigations carried out by Raman were experimental and theoretical studies on the diffraction of light by acoustic waves of ultrasonic and hypersonic frequencies (published 1934-1942), and those on the effects produced by X-rays on infrared vibrations in crystals exposed to ordinary light.

He also started a company called Travancore Chemical and Manufacturing Co. Ltd. in 1943 along with Dr. Krishnamurthy. The Company during its 60 year history established four factories in Southern India. In 1947, he was appointed as the first National Professor by the new government of Independent India. In 1948 Raman, through studying the spectroscopic behavior of crystals, approached in a new manner fundamental problems of crystal dynamics. He dealt with the structure and properties of diamond, the structure and optical behavior of numerous iridescent substances (labradorite, pearly feldspar, agate, opal, and pearls). Among his other interests were the optics of colloids, electrical and magnetic anisotropy, and the physiology of human vision. Raman retired from the Indian Institute of Science in 1948 and established the Raman Research Institute in Bangalore, Karnataka a year later. He served as its director and remained active there until his death in 1970, in Bangalore, at the age of 82. He was married on 6 May 1907 to Lokasundari Ammal with whom he had one son, Radhakrishnan.



"DEFEATIST SPIRIT MUST GO"

Hindu Monday 1941 1st December

SIR C. V. RAMAN'S ADVICE TO YOUTH

(ASSOCIATED PRESS OF INDIA.)

PATNA, Nov. 28.

Addressing the Patna University Convocation this evening in the Wheeler Senate House, Dr. Sachidanand Sinha, Vice-Chancellor, presiding, Sir C. V. Raman said:

"You, our young men, come to the Universities and leave them to face the world—a world which may seem to be an unsympathetic harsh world. I would like to tell the young men and women before me not to lose hope and courage. Success can only come to you by courageous devotion to the task lying in front of you, and there is nothing worth in this world that can come without the sweat of our brow. We have abundant human material in India. Speaking as a teacher of twenty-four years' experience, I can assert, without fear of contradiction, that the quality of the Indian mind is equal to the quality of any Teutonic, Nordic or Anglo-Saxon mind. What we lack is perhaps courage, what we lack is perhaps the driving force which takes one anywhere. We have, I think, developed an inferiority complex. I think what is needed in India to-day is the destruction of that defeatist spirit. We need a spirit of victory, a spirit that will carry us on to our rightful place under the sun, a spirit which will recognise that, we as inheritors of a proud civilisation, are entitled to a rightful place on this planet. If that indomitable spirit were to arise, nothing can hold us from achieving our rightful destiny."

Raman was honoured with a large number of honorary doctorates and memberships of scientific societies. He was elected a Fellow of the Royal Society early in his career (1924) and knighted in 1929. In 1930 he won the Nobel Prize in Physics. In 1954 he was awarded the Bharat Ratna. He was also awarded the Lenin Peace Prize in 1957. When the first President of India Dr. Rajendra Prasad wrote a letter to C.V Raman to visit India to receive India's Highest Civilian Honor Bharat Ratna in 1954,C.V Raman replied like this: "Dear Mr President, I thank you for giving me such a great honour, but I have a problem. I am guiding a scholar and he is submitting his thesis in December-January. I have to sign the thesis and won't be able to accept the invitation."

The Bharat Ratna, of course, was awarded to Dr Raman in absentia. This shows his attitude and his commitment for work. India celebrates National Science Day on 28 February of every year to commemorate the discovery of the Raman Effect in 1928.

## Raman Effect

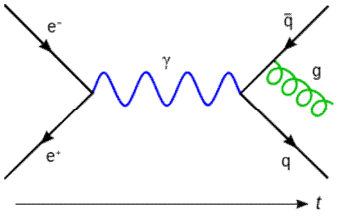
Raman scattering or the Raman effect is the inelastic scattering of a photon discovered by Sir Chandrasekhara Venkata Raman in liquids and by Grigory Landsberg and Leonid Mandelstam in crystals. When light is scattered from an atom or molecule, most photons are elastic scattering (Rayleigh scattering), such that the scattered photons have the same energy (frequency) and wavelength as the incident photons. However, a small fraction of the scattered light (approximately 1 in 10 million photons) is scattered by an excitation, with the scattered photons having a frequency different from, and usually lower than, the frequency of the incident photons. In a gas, Raman scattering can occur with a change in vibrational, rotational or electronic energy of a molecule. Chemists are concerned primarily with the vibrational Raman Effect. In 1922, Indian physicist C. V. Raman published his work on the "Molecular Diffraction of Light," the first of a series of investigations with his collaborators which ultimately led to his discovery (on 28 February 1928) of the radiation effect which bears his name. The Raman Effect was first reported by C. V. Raman and K. S. Krishnan, and independently by Grigory Landsberg and Leonid Mandelstam, in 1928. Raman received the Nobel Prize in 1930 for his work on the scattering of light. In 1998 the Raman Effect was designated an ACS National Historical Chemical Landmark in recognition of its significance as a tool for analyzing the composition of liquids, gases, and solids.

**Sir Chandrasekhara VenkataRaman**
**Born:** 7 November 1888, Tiruchirappalli, India
**Died:** 21 November 1970, Bangalore, India
**Affiliation at the time of the award:** Calcutta University, Calcutta, India
**Prize motivation:** "for his work on the scattering of light and for the discovery of the effect named after him"
**Field:** atomic physics, electromagnetism
**Prize share:** 1/1

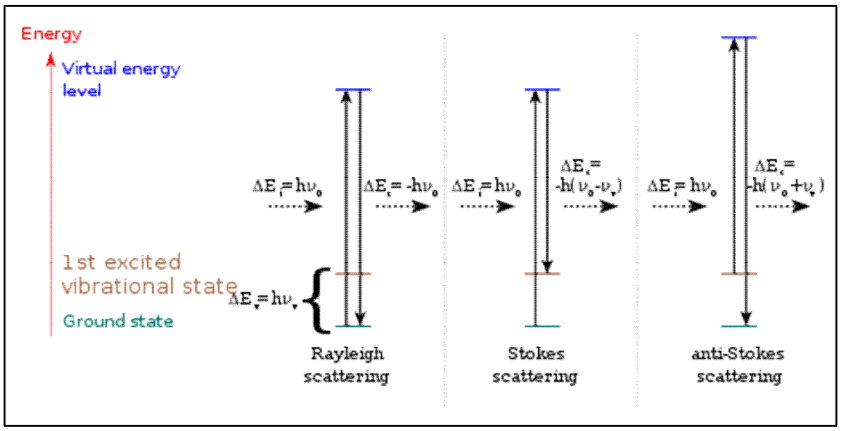
## Raman scattering

The different possibilities of visual light scattering: Rayleigh scattering (no Raman effect), Stokes scattering (molecule absorbs energy) and anti-Stokes scattering (molecule loses energy). There are two types of Raman scattering, Stokes scattering and anti-Stokes scattering.The interaction of light with matter in a linear regime allows the absorption or simultaneous emission of light precisely matching the difference in energy levels of the interacting electrons.The Raman Effect corresponds, in perturbation theory, to the absorption and subsequent emission of a photon via an intermediate electron state, having a virtual energy level (see Feynman diagram). There are three possibilities:

**Feynman diagram** →



- no energy exchange between the incident photons and the molecules (and hence no Raman effect)
- energy exchanges occur between the incident photons and the molecules. The energy differences are equal to the differences of the vibrational and rotational energy-levels of the molecule. In crystals only specific photons are allowed (solutions of the wave equations which do not cancel themselves) by the periodic structure, so Raman scattering can only appear at certain frequencies. In amorphous materials like glasses, more photons are allowed and thereby the discrete spectral lines become broad.
- molecule absorbs energy: Stokes scattering. The resulting photon of lower energy generates a Stokes line on the red side of the incident spectrum.
- molecule loses energy: anti-Stokes scattering. Incident photons are shifted to the blue side of the spectrum, thus generating an anti-Stokes line.




These differences in energy are measured by subtracting the energy of the mono energetic laser light from the energy of the scattered photons. The absolute value, however, doesn't depend on the process (Stokes or anti-Stokes scattering), because onlythe energy of the different vibrational levels is of importance. Therefore, the Raman spectrum is symmetric relative to the Rayleigh band. In addition, the intensities of the Raman bands are only dependent on the number of molecules occupying the different vibrational states, when the process began. If the sample is in thermal equilibrium, the relative numbers of molecules in states of different energy will be given by the Boltzmann distribution:

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} e^{-\frac{\Delta E_v}{kT}}$$ where:

$N0$: number of atoms in the lower vibrational state
$N1$: number of atoms in the higher vibrational state
$g0$: degeneracy of the lower vibrational state (number of orbitals of the same energy)
$g1$: degeneracy of the higher vibrational state
$\Delta Ev$: energy difference between these two vibrational states
$k$: Boltzmann's constant
$T$: temperature in kelvin

## Fluorescence effect of Light

The Raman Effect differs from the process of fluorescence. For the latter, the incident light is completely absorbed and the system is transferred to an excited state from which it can go to various lower states only after a certain resonance lifetime. The result of both processes is essentially the same: A photon with the frequency different from


that of the incident photon is produced and the molecule is brought to a higher or lower energy level. But the major difference is that the Raman Effect can take place for any frequency of the incident light. In contrast to the fluorescence effect, the Raman Effect is therefore not a resonant effect. In practice, this means that a fluorescence peak is anchored at a specific excitation frequency, whereas a Raman peak maintains a constant separation from the excitation frequency. Another, related distinction is that Raman scattering is a coherent process, whereas fluorescence is not. This means that the measured intensity is the square of a coherent sum of scattering amplitudes. In practice, this means that different paths to the excitation of the same mode may interfere, leading to Fano effects: asymmetries in the shape of the scattering peaks.

## Selection rules

The distortion of a molecule in an electric field, and therefore the vibrational Raman cross section, is determined by its polarizability. A Raman transition from one state to another, and therefore a Raman shift, can be activated optically only in the presence of non-zero polarizability derivative with respect to the normal coordinate (that is, the vibration or rotation):

$$\left|\frac{\partial \alpha}{\partial Q}\right| > 0$$

Raman-active vibrations/rotations can be identified by using almost any textbook that treats quantum mechanics or group theory for chemistry. Then, Raman-active modes can be found for molecules or crystals that show symmetry by using the appropriate character table for that symmetry group.

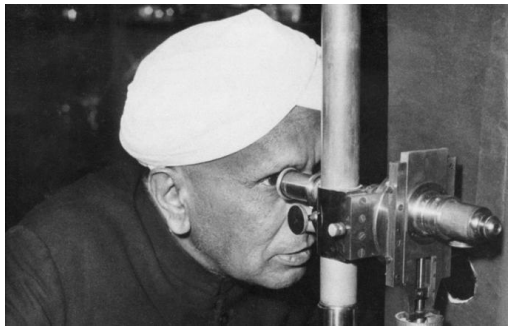
## Stimulated Raman Scattering (SRS)

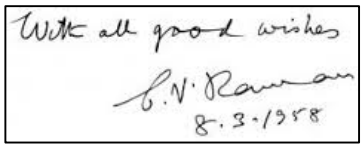
Raman amplification can be obtained by using Stimulated Raman Scattering (SRS), which actually is a combination of a Raman process with stimulated emission. It is interesting for application in telecommunication fibers to amplify inside the standard material with low noise for the amplification process. However, the process requires significant power and thus imposes more stringent limits on the material. The amplification band can be up to 100 nm broad, depending on the availability of allowed photon states.

## Spectra generation by Raman spectrograph

For high intensity CW (continuous wave) lasers, SRS can be used to produce broad bandwidth spectra. This process can also be seen as a special case of four wave mixing, where the frequencies of the two incident photons are equal and the emitted spectra are found in two bands separated from the incident light by the phonon energies. The initial Raman spectrum is built up with spontaneous emission and is amplified later on. At high pumping levels in long fibers, higher order Raman spectra can be generated by using the Raman spectrum as a new starting point, thereby building a chain of new spectra with decreasing amplitude. The disadvantage of intrinsic noise due to the initial spontaneous process can be overcome by seeding a spectrum at the beginning, or even using a feedback loop like in a resonator to stabilize the process. Since this technology easily fits into the fast evolving fiber laser field and there is demand for transversal coherent high intensity light sources (i.e. broadband telecommunication, imaging applications), Raman amplification and spectrum generation might be widely used in the near future.

## Applications of Raman spectroscopy






Raman spectroscopy employs the Raman Effect for materials analysis. The frequency of light scattered from a molecule may be changed based on the structural characteristics of the molecular bonds. A monochromatic light source (laser) is required for illumination, and a spectrogram of the scattered light then shows the deviations caused by state changes in the molecule. Raman spectroscopy is used as a tool to detect high-frequency phonons, magnons and electronic excitations. Raman spectroscopy is also used in combustion diagnostics. Being a completely non-intrusive technique, it permits the detection of the major species and temperature distribution inside combustors and in flames without any perturbation of the (mainly fluid dynamic and reactive) processes examined. Stimulated Raman transitions are also widely used for manipulating a trapped ion's energy levels, and thus basis qubit states. Raman amplification is used in optical amplifiers. Raman lidar is used in Atmospheric Physics to study aerosol vertical distribution.

## REFERENCES

1. Nityananda ,The Great Man & His Aberrations, Tehelka Magazine, Volume 8 Issue 48, Dated December 3, 2011.
2. Nobel Lectures (Physics) 1922-1941. Elsevier Publishing Company : New York 1965, pp 263-277
3. S.N. Sen. Prof. C.V. Raman : Scientific Work at Calcutta. Indian Association for the Cultivation of Science : Kolkata, 1988.
4. Anthony T. Tu. Raman Spectroscopy in Biology. John Wiley & Sons : New York, 1982.
5. Subodh Mahanti. Kariamanikkam Srinivasa Krishnan. Dream-2047, January 2002 New Delhi.
6. James H. Hibben. The Raman Effect and Its Chemical Applications. Reinhold Publishing Company : New York 1939.
7. G. Venkataraman. Journey Into Light : Life and Science of Raman. Indian Academy of Science : Bangalore, 1988,reprinted by Penguin Books India : New Delhi, 1994.
8. Nobel Lectures (Physics) 1922-1941. Elsevier Publishing Company : New York 1965, pp 263-277.
9. S.N. Sen. Prof. C.V. Raman : Scientific Work at Calcutta. Indian Association for the Cultivation of Science : Kolkata, 1988.
10. http://www.nobelprize.org/nobel_prizes/physics/laureates/1930/raman-bio.html .
11. http://www.iacs.res.in/noble-lecture_sir_cv_raman.pdf
12. Letters to Editor, C. V. Raman,The Colour of the Sea, Nature 108, 367-367 (17 November 1921)
13. www.vigyanprasar.gov.in/scientists/cvraman/raman1.html.
14. http://www.britannica.com/EBchecked/topic/490449/Sir-Chandrasekhara-Venkata-Raman
15. http://www.indianscience.in/info/index.php/CV_Raman.

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RESEARCH ARTICLE

# Evaluation of Antiarthritic activity of *Vitex altissima* L.f. Stem Bark.

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## ABSTRACT

Rheumatoid arthritis is a disease marked by chronic inflammation of the joints and involvement of the autoimmune system. It can affect the connective tissues of the joints, muscles as well as some organs of the body. For the treatment of Rheumatoid arthritis allopathic drugs are available for temporary relief and also with several side effects. In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions. A systemic study of anti-inflammatory effects of Indian medicinal plants began by Gujral and his associates in 1956 and they screened a number of plants for their anti-arthritic effects. Subsequently, various workers from different laboratories in India have made significant contribution. The anti-arthritic activity of the ethanolic extract of *Vitex altissima* stem bark was evaluated by Fruend's Complete Adjuant (FCA) arthritis model in wistar rats. Evaluate the levels of SOD, CAS, GST AND GPx which are involved in the destruction of the reactive oxygen intermediates and scavenging of free radicals in FCA induced rats. Oral administration of the ethanolic extract (at dose levels of 200 and 400mg/kg bw) exhibited significant inhibition of FCA induced paw edema. FCA induced animals showed significant increase in the serum lipid peroxidation when compared to the control animals. Concomitantly the activities of the antioxidant marker enzymes such as SOD, CAT, GPx and GST were decreased.The observed anti-arthritis activity of extract may be to the presence of phytoconstituents such as alkaloid and flavonoids.

**Keywords:** anti-arthritis, *Vitex altissima*, Fruend's Complete Adjuant, Ayurvedic, phytoconstituents. Rheumatoid arthritis.

# INTRODUCTION

Rheumatoid arthritis is a generalized disease affecting the connective tissues, of the whole body. Often it affects the joints in the fingers, wrists, knees and elbows. Rheumatoid arthritis is often triggered by an infection which compels the immune system to attack cells with antibodies. While the immune system is fighting the infection, it also attacks the tissues surrounding certain tissues, which causes them to deteriorate resulting in malfunctioning of joints. In this way, rheumatoid arthritis is considered as an autoimmune disease or a systematic illness because it can affect multiple or all areas of the body.As a chronic illness, the effects of rheumatoid arthritis can be suffered for years. Some people go years without experiencing any symptoms. However this disease is progressive and will eventually result in joint destruction and functional disability. In children, the disorder can present with a skin rash, fever, pain, disability and limitations in daily activities [1].

Basically a joint is where one bone moves on another bone. Ligaments hold the bones together. The ligaments are like elastic bands, while they keep the bones in place, the muscles relax and contract to make the joints move. Cartilage covers the bone surface to stop the two bones from rubbing directly against each other. The covering of cartilage allows the joints to work smoothly and painlessly. The space within joint-joint cavity has synovial fluid. Synovial fluid nourishes the joint and the cartilage. The synovial fluid is produced by the synovium (synovial membrane) which lines the joint cavity [2]. General drugs available for arthritis are three general classes of drugs commonly used in the treatment of rheumatoid arthritis: Non-Steroidal Anti- Inflammatory Drugs (NSAIDs), corticosteroids and Disease Modifying Anti-Rheumatic Drugs (DMARDs). NSAIDS and corticosteroids have a short onset of action while DMARDs can take several weeks or months to demonstrate a clinical effect [3]. Indomethacin (Indocid) has been used in the treatment of rheumatoid arthritis for over 10 years. Many patients get a worthwhile beneficial effect from it but a significant proportion of patients are not improved [4]. There is at present no clinical means of therapy with indomethacin, and possible reasons for failure to improve vary from a failure to take the capsules due to the presence of severe side effects. Side-effects occur in three major groups; referable to the central nervous system, the gastrointestinal system, and the skin.

*Vitex altissima* is also known as tall chaste tree belongs to family Verbenaceae. The plant is distributed throughout South India, in evergreen and deciduous forests and in Srilanka. It is common in forest, less on slopes, down to foothills, hills above 600m. It is a densely foliaceous tree with extensive crown. A large tree grows up to 40 meters in height. *Vitex* species have been reported for their anti-inflammatory activities and to name a few are *V. agnus-castus* [5], *V. doniana* [6], *V. glabrata* [7] and *V. trifolia* [8]. The present study was undertaken to evaluate anti-arthritis activity of ethanolic extract of *Vitex altissima* stembark in rats.

# MATERIALS AND METHODS

*Vitex altissima* L.f. Stem barks were collected during the month of May 2011 from Servarayan hills of Eastern ghats, Tamil Nadu, India. For *in vivo* studies, the shade dried and powdered stem bark material was extracted in soxhlet extractor with ethanol after dewaxing with petroleum ether. The extract was evaporated to remove even the final traces of ethanol. The dried extract was suspended in distilled water right before use.

## Experimental animals

Male wistar rats (120-150 g) and Swiss albino mice (25-30 g) used in the present study were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. The animals were housed in polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage under standard environmental conditions of temperature (23 ± 1°C), relative humidity (55 ± 1%), 12 h/12 h light/dark cycle and fed with standard pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted over night before the experimental schedule, but had free access for water *ad libitum*. The protocol for the present study was approved by Animal Ethics Committee in accordance with the guidelines for the care and use of laboratory animals set by CPCSEA.

### FCA induced Anti-arthritis

Wistar albino male rats (150-200 g) were divided into five groups of six animals each (n=6). Group I served as control. Arthritis was induced in rats by injecting 0.1 mL of 0.1% Freund's complete adjuvant (FCA) (Sigma Aldrich, USA) into the subplantar region in the right hind paw of group II – V rats on the first day of the experiment. Group III was administered with indomethacin (10 mg/ kg/day p.o.) daily for 15 days which served as the standard reference group [9]. Group IV and V were administered with ethanolic extract of *Vitex altissima* stem bark at the doses of 200 and 400 mg/kg/day p.o. respectively daily for 15 days. The increase in joint diameter was measured daily starting from day 1, by using vernier caliper.

At the end of experimental regimen, all the animals were subjected to chloroform anesthesia. Blood samples were collected by cardiac puncture and allowed to clot for 20-30 min and centrifuged in a refrigerated centrifuge (4°C) at 3000 rpm for 10 minutes. In serum, lipid peroxidation and the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase were determined using standard methods.

## RESULTS

### Anti-arthritic activity

The anti-arthritic activity of the ethanolic extract of *V. altissima* stem bark was studied against FCA-induced arthritis in rat model and the results are presented in Fig. 1, and Table 1 - 3.The tested drug extract at two different concentrations (200 and 400 mg/Kg b.w. p.o.) were administered orally and the activity was compared with the standard drug indomethacin (50 mg/kg b.w. p.o.). Subplantar injection of FCA (Freund's complete adjuvant) in the rat hind paw led to the development of arthritis. The standard indomethacin was found to inhibit this edema to an extent of 72.20%. The test extract, at the doses of 200 and 400 mg/kg, p.o. showed 54.15% and 68.27% inhibition respectively (Table 2).

### Effect of *V. altissima* stem bark on serum antioxidant enzymatic parameters

The levels of enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAS), glutathione-S-transferase (GST) and glutathione peroxidase (GPx), which are involved in the destruction of the reactive oxygen intermediates and scavenging of free radicals in FCA induced rats are presented in Table3. As a result of inflammation induced by FCA, the levels of SOD, CAT, GST and GPx, were decreased ($P$<0.001) in FCA induced group II animals when compared to control rats. After treatment with standard indomethacin and ethanolic extract of *V. altissima*, the levels of these enzymes were significantly increased ($P$<0.001) which revealed an increase in the profound activity of antioxidant enzymes when compared to the group II animals. This clearly indicates that upon treatment with the ethanolic extract at two different doses, (200 and 400mg/Kg bw p.o., respectively) the antioxidant levels were reverted back to near – normal levels in group IV and group V animals respectively when compared with group II animals. The higher dose of the plant extract, 400 mg/Kg b.w. showed activities similar to that of the standard reference drug.

### Effect of *V. altissima* stem bark on lipid peroxidation

The levels of lipid peroxidation in the serum which determines the extent of oxidative damage are presented in Table3. In the present study, lipid peroxidation levels were found to be significantly ($P$<0.001) increased in FCA induced group II animals when compared to the group I control animals. After administration of standard drug indomethacin and sample extract at two different doses (200 and 400mg/Kg b.w. p.o.,), the level of lipid peroxidation was found to be significantly decreased when compared to group II animals. The results revealed that on sample drug administration the levels of peroxide scavenging increased with the increasing concentration of the sample. The

ethanolic extract at a dose of 400mg/Kg b.w. exhibits an equivalent activity to standard drug indomethacin. This indicates that inflammation induced groups are subjected to oxidative stress while a statistically significant ($P$<0.001) reduction of lipid peroxide activity was observed in standard drug and sample treated group.

## DISCUSSION

Freund's Complete Adjuvant (FCA) induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis [10,11].Chronic inflammation in the FCA induced model is manifested as a progressive increase in the volume of the injected paw. In the present study, the anti-arthritic activity of the ethanolic extract of *V. altissima* stem bark at two different concentrations (200 and 400 mg/Kg bw p.o.) were tested against FCA induced arthritic model ( Fig. 1, and Table 1 -3). The arthritic rats showed soft tissue swelling around the ankle-joints during the acute phase of arthritis and it was considered to be edema of the particular tissues. The swelling was found to be increasing in the initial phase of inflammation and then reached a peak edema on day 8 of the injection. It is noteworthy that the inhibitory effect of the ethanolic extract of *V. altissima* stem bark (400 mg/kg bw p.o.) on the volume of the injected paw was comparable with that of the standard indomethacin (50 mg/kg bw p.o.). FCA-induced polyarthritis is associated with an immune-mediated inflammatory reaction and the rat is unique in developing polyarthritis after FCA treatment [12]. The initial reaction of edema and soft-tissue thickening at the depot site in this model is caused by the irritant effect of the adjuvant, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events [13]. In the groups treated with indomethacin and the ethanolic extract of *V. altissima* stem bark, the reduction in paw-swelling from 6$^{th}$ day onwards may be due to immunological protection rendered by them.

In arthritic condition, the granulocytes and macrophages accumulate in the affected area and produce large amounts of superoxide and hydrogen peroxide radicals [14] and the estimation of these active species in the arthritis induced experimental animals help in assessing the free radical scavenging property and indirectly the anti-arthritic potential of the ethanolic extract of *V. altissima* stem bark. In the present study, FCA induced animals showed significant increase in the levels of LPO (Table3) and with concomitant decrease in the activities of the antioxidant markers such as SOD, CAT, GPx and GST (Table3). The role of NO has been well established in an inflammatory response. As the inflammatory response progresses, large quantities of NO are generated through the induction of iNOS (inducible nitric oxide synthase) that reacts with superoxide anion to form peroxynitrate, a potent oxidizing molecule capable of eliciting lipid peroxidation. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids to form radical intermediates that bring about cellular damage. Malondialdehyde (MDA), a major end product of this reaction, is an index of lipid peroxidation and has been estimated as thiobarbituric acid reactive substance (TBARS) [15]. Besides, the infiltrating cells also generate reactive oxygen species and free radicals that bring about destruction of the inflamed joint. As a result, the scavenging enzyme SOD that leads to the formation of hydrogen peroxide is utilized and its activity is reduced in arthritic rats. The hydrogen peroxide thus generated is decomposed by catalase and glutathione peroxidase. Excessive production of lipid hydroperoxide may also contribute to decreased activity of GPx in arthritic condition. Administration of the ethanolic extract of *V. altissima* stem bark (200 and 400mg/kg bw) and the standard drug indomethacin (50 mg/kg bw) brought down the tissue levels of TBARS (Table3). Both the ethanolic extract of *V. altissima* stem bark and the standard drug indomethacin maintained the oxidative homeostasis, and the activities of SOD, catalase, GPx and GST were comparable to the control animals (Table3). The present study shows that the ethanolic extract of *V. altissima* stem bark markedly reduces oxidative stress associated with arthritic condition, and therefore has the potential to be used as an anti-arthritic agent.

## CONCLUSION

The anti-arthritic activity of the ethanolic extract of *V. altissima* stem bark was evaluated by Fruend's Complete Adjuant (FCA) arthritis model in wistar rats. Oral administration of the ethanolic extract (at dose levels of 200 and 400mg/kg bw) exhibited significant inhibition of FCA induced paw edema. FCA induced animals showed significant increase in the serum lipid peroxidation when compared to the control animals. Concomitantly the activities of the

antioxidant marker enzymes such as SOD, CAT, GPx and GST were decreased. The recovery of all the above said biochemical parameters by treatment of the experimental animals with the ethanolic extract of *V. altissima* stem bark at the dose of 400 mg/kg was almost similar to the activity shown by the standard drug indomethacin (50 mg/kg).These findings could justify, at least partially, the ethnomedicinal use of this plant in the management of pain, inflammation, and arthritis. Further studies are warranted for the isolation and identification of bioactive compounds and their mechanism of action as a phytodrug.

## REFERENCES

1) Verstappen, S.M., Bijlsma, J.W. and Verklejj, H., 2004. Overview of work disability in rheumatoid arthritis patients as observed in cross-sectional and longitudinal surveys. *Arthritis Rheum*, 5: 488-497.
2) Chan, K.W.A., Felson, D., Yood, R., 1994. The lag-time between onset of symptoms and diagnosis of rheumatoid arthritis. *Arthritis Rheum*, 37: 814-820.
3) Wright, D., Chapman, N., Foundling-Miah. 2006. Consensus guidelines on the medication management of adults with swallowing difficulties. *J Toxicology Sci*, 3:45-54.
4) Broll, H., Tausch, G., and Eberl, R. 1976. Long-term treatment of arthritis with indomethacin. In Inflammatory Arthropathies, 175-178.
5) Ahmad, Sadiq Azam, Shumaila Bashir, Ibrar Khan, Achyut Adhikari, Muhammad Iqbal Choudhary · 2010. Anti-inflammatory and enzyme inhibitory activities of a crude extract and a pterocarpan isolated from the aerial parts of *Vitex agnus-castus. Journal of Biotechnology*, 5(11): 1207-1215.
6) Ochieng, Charles O., Ismail O., Ishola, Sylvia A., Opiyo, Lawrence A.O.,Manguro1, Philip O., Owuor, Keng-Chong Wong. 2013. Phytoecdysteroids from the stem bark of vitex doniana and their anti-inflammatory effect. *Planta Med*, 79(1): 9-52.
7) Chouhan, C. S., Sridevi, K., Singh, N. K., Singh, S. K. 2012. Anti-inflammatory activity of ethanol extract of *Vitex glabrata* leaves. *Pak J Pharm Sci*, 25(1): 131-134.
8) Matsui, M., Adib-Conquy, M., Coste, A., Kumar-Roiné, S., Pipy, B., Laurent, D., Pauillac, S. 2012. Aqueous extract of Vitex trifolia L. (Labiatae) inhibits LPS-dependent regulation of inflammatory mediators in RAW 264.7 macrophages through inhibition of Nuclear Factor kappa B translocation and expression. *Journal of ethanopharmacology*, 143(1):24-32.
9) Prakash Babu N., Pandikumar P., Ignacimuthu S. 2009. Anti-inflammatory activity of *Albizia lebbeck* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. *Journal of Ethnopharmacology*, 125: 356–360.
10) Shinde, U. A., Phadake, A. S., Nair, A. M., Mungantiwar, A. A., Dishit, V. J., Saraf, M. N. 1999. Studies on the anti-inflammatory and analgesic activity of *Cedrus deodara* (Roxb.) Loud. wood oil. *Journal of Ethnopharmacology*, 65: 21–7.
11) Tsai, C. C., Lin, C. C. 1999. Anti-inflammatory effects of Taiwan folk medicine 'Teng- Khaia-U' on carrageenan and adjuvant-induced paw edema in rats. *Journal of Ethnopharmacology*, 64: 85–9.
12) Cai, X., Wong, Y. F., Zhou, H., Xie, Y., Liu, Z. Q., Jiang, Z. H., Bian, Z. X., Xu, H.X., Liu, L. 2006. The comparative study of Sprague-Dawley and Lewis rats in adjuvant induced arthritis. Naunyn-Schmiedeberg's Archives of Pharmacology, 373: 140–147.
13) Ward, J. R., Cloud, R. S. 1965. Comparative effect of antirheumatic drugs on adjuvant induced polyarthritis in rats. *Journal of Pharmacology and Experimental Therapeutics*,152: 116–121
14) Halley, A. E., Cheeseman, K. H. 1993. Measuring free radical reactions *in vivo*. *British Medical Bulletin*, 49: 494–505.
15) Kohen, R. and Nyska, A., 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*, 30: 620–650.

**Table1. Effect of ethanolic extract of *Vitex altissima* stem bark on paw volume against FCA induced arthritis.**

| Days | Paw volume (mm) | | | | |
|---|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV | Group V |
| 0 | 3.28 ± 0.16 | 3.15 ± 0.12 | 3.40 ± 0.08$^{**}$ | 3.42 ± 0.13$^{**}$ | 3.56 ± 0.03$^{***}$ |
| 1 | 3.28 ± 0.16 | 6.96 ± 0.24$^{\#\#\#}$ | 6.60 ± 0.21$^{**}$ | 7.29 ± 0.08$^{**}$ | 7.40 ± 0.20$^{***}$ |
| 2 | 3.28 ± 0.16 | 7.45 ± 0.23$^{\#\#\#}$ | 7.27 ± 0.14 | 7.95 ± 0.09$^{***}$ | 7.75 ± 0.14$^{**}$ |
| 3 | 3.28 ± 0.16 | 7.95 ± 0.02$^{\#\#\#}$ | 7.83 ± 0.06$^{*}$ | 8.23 ± 0.08$^{***}$ | 8.21 ± 0.09$^{***}$ |
| 4 | 3.28 ± 0.16 | 8.60 ± 0.09$^{\#\#\#}$ | 8.38 ± 0.09$^{**}$ | 8.88 ± 0.09$^{**}$ | 8.78 ± 0.07$^{**}$ |
| 5 | 3.28 ± 0.16 | 9.32 ± 0.05$^{\#\#\#}$ | 8.16 ± 0.04$^{***}$ | 9.20 ± 0.09 | 8.61 ± 0.05$^{**}$ |
| 6 | 3.28 ± 0.16 | 9.92 ± 0.04$^{\#\#\#}$ | 7.89 ± 0.05$^{***}$ | 9.66 ± 0.07$^{***}$ | 8.42 ± 0.11$^{***}$ |
| 7 | 3.28 ± 0.16 | 10.25 ± 0.09$^{\#\#\#}$ | 7.73 ± 0.11$^{***}$ | 9.34 ± 0.13$^{***}$ | 8.20 ± 0.12$^{***}$ |
| 8 | 3.28 ± 0.16 | 10.77 ± 0.04$^{\#\#\#}$ | 7.23 ± 0.06$^{***}$ | 9.09 ± 0.08$^{***}$ | 7.97 ± 0.08$^{***}$ |
| 9 | 3.28 ± 0.16 | 11.44 ± 0.08$^{\#\#\#}$ | 6.89 ± 0.10$^{***}$ | 8.69 ± 0.19$^{***}$ | 7.70 ± 0.11$^{***}$ |
| 10 | 3.28 ± 0.16 | 11.85 ± 0.06$^{\#\#\#}$ | 6.52 ± 0.12$^{***}$ | 8.13 ± 0.10$^{***}$ | 7.44 ± 0.08$^{***}$ |
| 11 | 3.28 ± 0.16 | 11.23 ± 0.07$^{\#\#\#}$ | 6.15 ± 0.05$^{***}$ | 7.65 ± 0.14$^{***}$ | 7.14 ± 0.07$^{***}$ |
| 12 | 3.28 ± 0.16 | 10.73 ± 0.06$^{\#\#\#}$ | 5.73 ± 0.06$^{***}$ | 7.03 ± 0.05$^{***}$ | 6.82 ± 0.05$^{***}$ |
| 13 | 3.28 ± 0.16 | 10.11 ± 0.04$^{\#\#\#}$ | 5.43 ± 0.06$^{***}$ | 6.90 ± 0.12$^{***}$ | 6.05 ± 0.04$^{***}$ |
| 14 | 3.28 ± 0.16 | 9.85 ± 0.08$^{\#\#\#}$ | 5.25 ± 0.08$^{***}$ | 6.58 ± 0.18$^{***}$ | 5.82 ± 0.06$^{***}$ |
| 15 | 3.28 ± 0.16 | 9.31 ± 0.08$^{\#\#\#}$ | 5.11 ± 0.04$^{***}$ | 6.24 ± 0.12$^{***}$ | 5.51 ± 0.12$^{***}$ |

Values are expressed as mean ± SD (n=6).,

$^{\#}$Variations in paw volume at $P<0.05$ when group II compared to group I, $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$.

Variations in paw volume at $P<0.05$ when group III – V compared to group II, $^{**}$ $P<0.01$, $^{***}$ $P<0.001$.

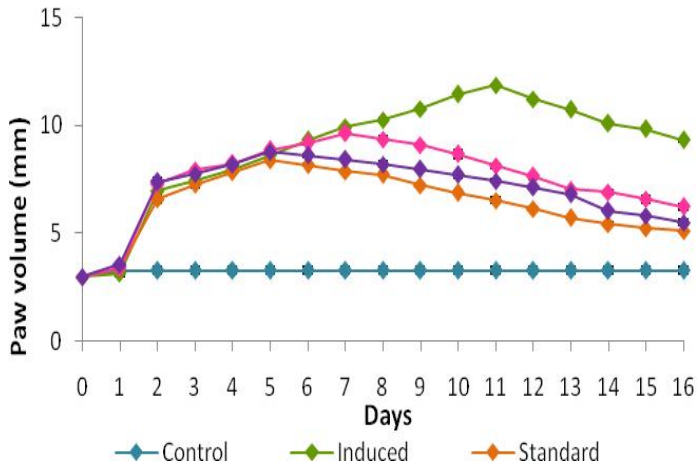


**Fig. 1. Effect of ethanolic extract of *Vitex altissima* stem bark and indomethacin on FCA induced arthritis.**

**Table 2. Effect of ethanolic extract of *Vitex altissima* stem bark on percentage protection against FCA induced arthritis.**

| Group | Dose (p.o.) | Initial paw volume (mm) | Final paw volume (mm) | Difference (mm) | Percentage protection (%) |
|---|---|---|---|---|---|
| **Control** | - | 3.28 ± 0.16 | 3.28 ± 0.16 | ----- | ----- |
| **Induced** | - | 3.15 ± 0.12 | 9.30 ± 0.08$^{\#\#\#}$ | 6.15 ± 0.19$^{\#\#\#}$ | ----- |
| **Indomethacin** | 10 mg/kg | 3.40 ± 0.08$^{**}$ | 5.11 ± 0.05$^{***}$ | 1.71 ± 0.09$^{***}$ | 72.20 |
| ***Vitex altissima*** | 200 mg/kg | 3.42 ± 0.13$^{**}$ | 6.24 ± 0.12$^{***}$ | 2.82 ± 0.25$^{***}$ | 54.15 |
| ***Vitex altissima*** | 400 mg/kg | 3.56 ± 0.03$^{***}$ | 5.51 ± 0.12$^{***}$ | 1.95 ± 0.12$^{***}$ | 68.27 |

Values are expressed as mean ± SD (n=6).
$^{\#}$Variations in paw volume at $P<0.05$ when group II compared to group I, $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$.
$^{*}$Variations in paw volume at $P<0.05$ when group III – V compared to group II, $^{**}$ $P<0.01$, $^{***}$ $P<0.001$.

**Table3. Effect of extract of *Vitex altissima* stem bark on serum antioxidant markers against FCA induced arthritis.**

| Group | Dose (p.o.) | SOD | CAT | GPx | GST | LPO |
|---|---|---|---|---|---|---|
| **Control** | - | 1.66 ± 0.02 | 9.68 ± 0.12 | 48.35 ± 0.38 | 15.25 ± 0.17 | 10.18 ± 0.16 |
| **Induced** | - | 0.92 ± 0.05$^{\#\#\#}$ | 3.40 ± 0.14$^{\#\#\#}$ | 20.57 ± 0.56$^{\#\#\#}$ | 7.34 ± 0.13$^{\#\#\#}$ | 25.35 ± 0.10$^{\#\#\#}$ |
| **Indomethacin** | 10 mg/kg | 1.63 ± 0.02$^{***}$ | 8.49 ± 0.17$^{***}$ | 45.35 ± 0.36$^{***}$ | 13.57 ± 0.12$^{***}$ | 10.41 ± 0.18$^{***}$ |
| ***Vitex altissima*** | 200 mg/kg | 1.46 ± 0.01$^{***}$ | 5.60 ± 0.14$^{***}$ | 39.28 ± 0.54$^{***}$ | 10.21 ± 0.08$^{***}$ | 17.15 ± 0.14$^{***}$ |
| ***Vitex altissima*** | 400 mg/kg | 1.51 ± 0.01$^{***}$ | 8.25 ± 0.06$^{***}$ | 41.24 ± 0.59$^{***}$ | 13.14 ± 0.08$^{***}$ | 11.18 ± 0.09$^{***}$ |

Values are expressed as mean ± SD (n=6). $^{\#}$Variations in paw volume at $P<0.05$ when group II compared to group I, $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$; $^{*}$Variations in paw volume at $P<0.05$ when group III – V compared to group II, $^{**}$ $P<0.01$, $^{***}$ $P<0.001$. SOD – Superoxide dismutase (Units/min/mg protein), CAT – Catalase (μ moles of $H_2O_2$ consumed/min/mg protein), GPx – Glutathione peroxidase (μ moles of GSH oxidized/min/mg protein) ,GST – Glutathione-S-transferase (μ moles of CDNB conjugation formed/min/mg protein), LPO – Lipid peroxidation (μ moles/mg protein).

**INSTRUCTION TO AUTHOR (S)**

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**Articles in Journals**

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.
   List the first six contributors followed by *et al*.
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

**Books and other Monographs**

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

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